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(54) Title: HUMAN EPENDYMIN

(57) Abstract

The present invention relates to a novel human Ependymin protein which is a member of the ependymin family. In particular, isolated nucleic acid molecules are provided encoding the human Ependymin protein. Ependymin polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of Ependymin activity. Also provided are diagnostic methods for detecting nervous system–related disorders and therapeutic methods for treating nervous system–related disorders.

Human Ependymin

Field of the Invention

The present invention relates to a novel human gene encoding a polypeptide which is a
5   member of the Ependymin family. More specifically, isolated nucleic acid molecules are
provided encoding a human polypeptide named Ependymin. Ependymin polypeptides are also
provided, as are vectors, host cells and recombinant methods for producing the same. Also
provided are diagnostic methods for detecting disorders related to the nervous system, and
therapeutic methods for treating such disorders.

10   The invention further relates to screening methods for identifying agonists and
antagonists of Ependymin activity.

Background of the Invention

Within the last several years, a number of ependymins have been molecularly cloned
from a variety of teleost fish including *Oncorhynchus mykiss* (rainbow trout; Muller-Schmid. A.,
15   *et al., Gene* **118**:189-196 (1992)), *Salmo salar* (Atlantic salmon; Muller-Schmid, A., *et al.,
Gene* **118**:189-196 (1992)), *Esox lucius* (pike; Muller-Schmid, A., *et al., J. Molec. Evol.*
36:578-585 (1993)), *Carassius auratus* (goldfish; Konigstorfer, A., *et al., J. Neurochem.*
52:310-312 (1989); Konigstorfer, A., *et al., J. Biol. Chem.* **264**:13689-13692 (1989)),
Brachydanio rerio (zebrafish; Sterrer, S., *et al., Neurosci.* **37**:277-284 (1990)), and *Clupea
20   harengus* (herring, Muller-Schmid, *et al., J. Molec. Evol.* **36**:578-585 (1993)). The
ependymins produced by these organisms are synthesized as precursors which contain
N-terminal, hydrophobic signal sequences. Each of these molecules contains multiple N-linked
glycosylation sites, only some of which are conserved between species (Schmidt, R. and
Shashoua, V. E. *J. Neurochem.* **36**:1368-1377 (1981); Schmidt, R. and Shashoua, V. E. *J.*
25   *Neurochem.* **40**:652-660 (1983); Ganb, B. and Hoffman, W. *Eur. J. Biochem.* **217**:275-280
(1993)). The precursor ependymins range in apparent molecular mass from 23.7 to 24.5 kDa,
while the secreted mature forms of these molecules are typically 21.6-22.3 kDa in size.

The piscine ependymins characterized thus far may be categorized according to the
number of cysteine residues present in the mature polypeptide. Mature salmoniform (*O. mykiss,*
30   *S. salar,* and *E. lucius*) ependymin polypeptides contain only four cysteine residues, whereas
mature cypriniform (*C. auratus* and *B. rerio*) and clupeiform (*C. harengus*) ependymin
polypeptides contain five and six cysteine residues, respectively (Hoffmann, W. *Int. J. Biochem.*
26:607-619 (1994)). Correspondingly, disulfide-linked dimerization of the salmoniform
ependymin polypeptides is not observed after non-reducing SDS-PAGE. However, cypriniform
35   and clupeiform ependymins are observed as disulfide-linked dimers under non-reducing
conditions. It is speculated that the dimerization occurs via the cysteine residue conserved only
between the salmoniform ependymins (this cysteine residue aligns with the lysine residue at
location 133 of human ependymin of the present invention as shown in SEQ ID NO:2).

Several lines of evidence have provided the basis for an understanding of the functional
role(s) of the ependymins. Ca^{2+}-binding has been demonstrated for at least goldfish and
rainbow trout ependymins (Schmidt, R. and Makiola, E. *Neuro. Chem. (Life Sci. Adv.)*
10:161-171 (1991); Ganb, B. and Hoffman, *supra*). Further, ependymins are the primary
5   cerebrospinal fluid component in a number of teleost fish (Schmidt, R. and Lapp, H.
Neurochem. Int. **10:**383-390 1987;). Finally, roughly two-thirds of Ca2+ in the CSF of
rainbow trout is protein-bound (Ganb, B. and Hoffman, *supra*). As a result, it is thought that
ependymins may function in Ca^{2+} homeostasis of the teleost piscine brain (Hoffman, W., *supra*).

In situ hybridization analyses have shown that ependymins are apparently synthesized
10   exclusively in miningeal fibroblasts of the mininx (also termed the endomeninx or leptomeninx)
of teleost fish (Konigstorfer, A., *et al.*, *Cell Tissue Res.* **261:**59-64 (1990)). Ependymins have
also been found to associate with collgen fibrils of the extracellular matrix (ECM; Schwarz, H., *et
al. Cell Tissue Res.* **273:**417-425 (1993)), and, further, have the capacity to serve as a substrate
for outgrowing retinal axons (Schmidt, J. T., *et al.*, *J. Neurobiol.* **22:**40-54 (1991)).

15   An additional role for ependymins has been identified in the field of learning and
memory. Using an experimental approach in which goldfish learn to swim to a specific
compartment of its environment to avoid an electric shock, investigators have determined that
the amount of unbound or unincorporated extracellular ependymins decreases after learning
(Piront, M. -L., and Schmidt, R. *Brain Res.* **442:**53-62 (1988); Schmidt, R. *J. Neurochem.*
20   **48:**1870-1878 (1987)). Further, blockage of functional ependymin molecules, either with
antibodies or antisense polynucleotides, resulted in the reversible inability of the experimental
animal to remember the task which it had learned. Removal of the inhibitory substance then
resulted in a reappearance of the learned ability (Schmidt, R. J. *supra*; Shashoua, V. E. and
Moore, M. E. *Brain Res.* **148:**441-449 (1978)).

25   Thus, there is a need for polypeptides that function as neurotrophic factors in the
regeneration of the optic and other nerves and in long-term memory consolidation, since
disturbances of such regulation may be involved in disorders relating to the complex molecular
and cellular process regulating neuronal and nervous system function. Such disorders may
include Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, pain, stroke,
30   depression, anxiety, epilepsy, and other neurological and psychiatric disorders. Therefore, there
is a need for identification and characterization of such human polypeptides which can play a
role in detecting, preventing, ameliorating or correcting such disorders.

Summary of the Invention

The present invention provides isolated nucleic acid molecules comprising a
35   polynucleotide encoding at least a portion of the Ependymin polypeptide having the complete
amino acid sequence shown in SEQ ID NO:2 or the complete amino acid sequence encoded by
the cDNA clone deposited as plasmid DNA as ATCC Deposit Number 209464 on November 14,
1997, at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia
20110-2209. The nucleotide sequence determined by sequencing the deposited Ependymin
40   clone, which is shown in Figures 1A, 1B, and 1C (SEQ ID NO:1), contains an open reading

frame encoding a complete polypeptide of 224 amino acid residues, including an initiation
codon encoding an N-terminal methionine at nucleotide positions 296-298, and a predicted
molecular weight of about 25.4 kDa. Nucleic acid molecules of the invention include those
encoding the complete amino acid sequence excepting the N-terminal methionine shown in SEQ
5   ID NO:2, or the complete amino acid sequence excepting the N-terminal methionine encoded
by the cDNA clone in ATCC Deposit Number 209464, which molecules also can encode
additional amino acids fused to the N-terminus of the Ependymin amino acid sequence.

The encoded polypeptide has a predicted leader sequence of 37 amino acids underlined
in Figures 1A, 1B, and 1C; and the amino acid sequence of the predicted mature Ependymin
10   protein is also shown in Figures 1A, 1B, and 1C, as amino acid residues 38-224 and as residues
1-187 in SEQ ID NO:2.

Thus, one aspect of the invention provides an isolated nucleic acid molecule comprising
a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a
nucleotide sequence encoding the Ependymin polypeptide having the complete amino acid
15   sequence in SEQ ID NO:2 (i.e., positions -37 to 187 of SEQ ID NO:2); (b) a nucleotide
sequence encoding the Ependymin polypeptide having the complete amino acid sequence in
SEQ ID NO:2 excepting the N-terminal methionine (i.e., positions -36 to 187 of SEQ ID NO:2);
(c) a nucleotide sequence encoding the predicted mature Ependymin polypeptide having the
amino acid sequence at positions 1 to 187 in SEQ ID NO:2; (d) a nucleotide sequence encoding
20   the Ependymin polypeptide having the complete amino acid sequence encoded by the cDNA
clone contained in ATCC Deposit No. 209464; (e) a nucleotide sequence encoding the
Ependymin polypeptide having the complete amino acid sequence excepting the N-terminal
methionine encoded by the cDNA clone contained in ATCC Deposit No. 209464; (f) a
nucleotide sequence encoding the mature Ependymin polypeptide having the amino acid
25   sequence encoded by the cDNA clone contained in ATCC Deposit No. 209464; and (g) a
nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e) or
(f), above.

Further embodiments of the invention include isolated nucleic acid molecules that
comprise a polynucleotide having a nucleotide sequence at least 90% identical, and more
30   preferably at least 95%, 96%, 97%, 98% or 99% identical, to any of the nucleotide sequences in
(a), (b), (c), (d), (e), (f) or (g), above, or a polynucleotide which hybridizes under stringent
hybridization conditions to a polynucleotide in (a), (b), (c), (d), (e), (f) or (g), above. This
polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to
a polynucleotide having a nucleotide sequence consisting of only A residues or of only T
35   residues.

An additional nucleic acid embodiment of the invention relates to an isolated nucleic
acid molecule comprising a polynucleotide which encodes the amino acid sequence of an
epitope-bearing portion of a Ependymin polypeptide having an amino acid sequence in (a), (b),
(c), (d), (e) or (f), above. A further nucleic acid embodiment of the invention relates to an
40   isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid
sequence of a Ependymin polypeptide having an amino acid sequence which contains at least

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one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, even more preferably, not more than 40 conservative amino acid substitutions, still more preferably, not more than 30 conservative amino acid substitutions, and still even more preferably, not more than 20 conservative amino acid substitutions. Of course, in order of

5   ever-increasing preference, it is highly preferable for a polynucleotide which encodes the amino acid sequence of a Ependymin polypeptide to have an amino acid sequence which contains not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant

10   vectors, as well as to methods of making such vectors and host cells and for using them for production of Ependymin polypeptides or peptides by recombinant techniques.

The invention further provides an isolated Ependymin polypeptide comprising an amino acid sequence selected from the group consisting of: (a) the amino acid sequence of the full-length Ependymin polypeptide having the complete amino acid sequence shown in SEQ ID

15   NO:2 (i.e., positions -37 to 187 of SEQ ID NO:2); (b) the amino acid sequence of the full-length Ependymin polypeptide having the complete amino acid sequence shown in SEQ ID NO:2 excepting the N-terminal methionine (i.e., positions -36 to 187 of SEQ ID NO:2); (c) the amino acid sequence of the predicted mature Ependymin polypeptide having the amino acid sequence at positions 1 to 187 in SEQ ID NO:2; (d) the complete amino acid sequence encoded

20   by the cDNA clone contained in the ATCC Deposit No. 209464; (e) the complete amino acid sequence excepting the N-terminal methionine encoded by the cDNA clone contained in the ATCC Deposit No. 209464; and (f) the complete amino acid sequence of the predicted mature Ependymin polypeptide encoded by the cDNA clone contained in the ATCC Deposit No. 209464. The polypeptides of the present invention also include polypeptides having an amino

25   acid sequence at least 80% identical, more preferably at least 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those described in (a), (b), (c), (d), (e) or (f), above, as well as polypeptides having an amino acid sequence with at least 90% similarity, and more preferably at least 95% similarity, to those above.

An additional embodiment of this aspect of the invention relates to a peptide or

30   polypeptide which comprises the amino acid sequence of an epitope-bearing portion of a Ependymin polypeptide having an amino acid sequence described in (a), (b), (c), (d), (e) or (f), above. Peptides or polypeptides having the amino acid sequence of an epitope-bearing portion of a Ependymin polypeptide of the invention include portions of such polypeptides with at least six or seven, preferably at least nine, and more preferably at least about 30 amino acids to about

35   50 amino acids, although epitope-bearing polypeptides of any length up to and including the entire amino acid sequence of a polypeptide of the invention described above also are included in the invention.

A further embodiment of the invention relates to a peptide or polypeptide which comprises the amino acid sequence of a Ependymin polypeptide having an amino acid sequence

40   which contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, even more preferably, not more than 40 conservative

amino acid substitutions, still more preferably, not more than 30 conservative amino acid substitutions, and still even more preferably, not more than 20 conservative amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid

5   sequence of a Ependymin polypeptide, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

In another embodiment, the invention provides an isolated antibody that binds specifically to a Ependymin polypeptide having an amino acid sequence described in (a), (b), (c), (d), (e) or (f) above. The invention further provides methods for isolating antibodies that

10   bind specifically to a Ependymin polypeptide having an amino acid sequence as described herein. Such antibodies are useful diagnostically or therapeutically as described below.

The invention also provides for pharmaceutical compositions comprising Ependymin polypeptides, particularly human Ependymin polypeptides, which may be employed, for instance, to treat Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, pain,

15   stroke, depression, anxiety, epilepsy, and other neurological and psychiatric disorders. Methods of treating individuals in need of Ependymin polypeptides are also provided.

The invention further provides compositions comprising a Ependymin polynucleotide or an Ependymin polypeptide for administration to cells *in vitro*, to cells *ex vivo* and to cells *in vivo*, or to a multicellular organism. In certain particularly preferred embodiments of this aspect

20   of the invention, the compositions comprise a Ependymin polynucleotide for expression of a Ependymin polypeptide in a host organism for treatment of disease. Particularly preferred in this regard is expression in a human patient for treatment of a dysfunction associated with aberrant endogenous activity of a Ependymin

In another aspect, a screening assay for agonists and antagonists is provided which

25   involves determining the effect a candidate compound has on Ependymin binding to a receptor. In particular, the method involves contacting the receptor with a Ependymin polypeptide and a candidate compound and determining whether Ependymin polypeptide binding to the receptor is increased or decreased due to the presence of the candidate compound. In this assay, an increase in binding of Ependymin over the standard binding indicates that the candidate

30   compound is an agonist of Ependymin binding activity and a decrease in Ependymin binding compared to the standard indicates that the compound is an antagonist of Ependymin binding activity.

In yet another aspect, the Ependymin may bind to a cell surface protein which also function as a viral receptor or coreceptor. Thus, Ependymin, or agonists or antagonists thereof,

35   may be used to regulate viral infectivity at the level of viral binding or interaction with the Ependymin receptor or coreceptor or during the process of viral internalization or entry into the cell.

It has been discovered that Ependymin is expressed not only in primary dendritic cells, but also in the KMH2 cell line, placenta, fetal and adult liver, spinal cord, osteoclastoma,

40   cerebellum, synovial fibroblasts, 12 week old early stage human embryo, adrenal gland tumor, whole brain, Hodgkin's Lymphoma tissue, macrophages, HEL cell line, and chondrosarcoma.

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Therefore, nucleic acids of the invention are useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). In addition, for a number of

5   disorders of the above tissues or cells, particularly of the nervous system, significantly higher or lower levels of Ependymin gene expression may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" Ependymin gene expression level, i.e., the Ependymin expression level in healthy tissue from an individual not

10 having the nervous system disorder. Thus, the invention provides a diagnostic method useful during diagnosis of such a disorder, which involves: (a) assaying Ependymin gene expression level in cells or body fluid of an individual; (b) comparing the Ependymin gene expression level with a standard Ependymin gene expression level, whereby an increase or decrease in the assayed Ependymin gene expression level compared to the standard expression level is

15 indicative of disorder in the nervous system.

An additional aspect of the invention is related to a method for treating an individual in need of an increased level of Ependymin activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an isolated Ependymin polypeptide of the invention or an agonist thereof.

20 A still further aspect of the invention is related to a method for treating an individual in need of a decreased level of Ependymin activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of an Ependymin antagonist. Preferred antagonists for use in the present invention are Ependymin-specific antibodies.

25 *Brief Description of the Figures*

Figures 1A, 1B, and 1C show the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of Ependymin. The predicted leader sequence of about 37 amino acids is underlined. Note that the methionine residue at the beginning of the leader sequence in Figures 1A, 1B, and 1C is shown in position number (positive) 1, whereas the leader

30 positions in the corresponding sequence of SEQ ID NO:2 are designated with negative position numbers. Thus, the leader sequence positions 1 to 37 in Figures 1A, 1B, and 1C correspond to positions -37 to -1 in SEQ ID NO:2.

Two potential asparagine-linked glycosylation sites are marked in the amino acid sequence of Ependymin. The sites are asparagine-130 and asparagine-182 in Figures 1A, 1B,

35 and 1C (asparagine-93 and asparagine-145 in SEQ ID NO:2), and are labeled with the bold pound symbol (#) above the nucleotide sequence coupled with a bolded one letter abbreviation for the asparagine (N) in the amino acid sequence in Figures 1A, 1B, and 1C; that is, the actual asparagine residues which are potentially glycosylated is bolded in Figures 1A, 1B, and 1C.

A polyadenylation signal sequence is present in the 3' untranslated region of the

40 nucleotide sequence of Ependymin of the present invention. The polyadenylation signal

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sequence is delineated by a double underline in the Ependymin sequence shown in Figures 1A, 1B, and 1C.

Regions of high identity between Human Ependymin of the present invention and several previously identified piscine ependymins (an alignment of these sequences is presented in Figure 2) are delineated in Figures 1A, 1B, and 1C with a double underline. These regions are not limiting and are labeled as Conserved Domain (CD)-II, CD-III, CD-IV, CD-V, CD-VI, CD-VII, CD-IX, and CD-X in Figures 1A, 1B, and 1C.

Figure 2 shows the regions of identity between the amino acid sequences of the Ependymin protein and translation product of the Zebrafish mRNA for Ependymin (SEQ ID NO:6), determined by the computer program Bestfit (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711) using the default parameters.

Figures 3A and 3B are a multiple sequence alignment illustrating a comparison of the amino acid sequences of the novel human ependymin of the present invention (SEQ ID NO:2) and five previously described ependymin-like molecules. Conserved residues are highlighted in black. The ependymin homologues shown in the figure are goldfish ependymin II (SEQ ID NO:3; GenBank Accession No. J04986), rainbow trout ependymin II (SEQ ID NO:4; GenBank Accession No. M93698), common carp ependymin (SEQ ID NO:5; GenBank Accession No. U00432), zebrafish ependymin (SEQ ID NO:6; GenBank Accession No. X52502), and Atlantic herring ependymin (SEQ ID NO:7; GenBank Accession No. L09065).

Figure 4 shows an analysis of the Ependymin amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index or Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of the Ependymin protein, i.e., regions from which epitope-bearing peptides of the invention can be obtained.

The data presented in Figure 4 are also represented in tabular form in Table I. The columns are labeled with the headings "Res", "Position", and Roman Numerals I-XIV. The column headings refer to the following features of the amino acid sequence presented in Figure 4 and Table I: "Res": amino acid residue of Figures 1A, 1B, and 1C (which is the identical sequence shown in SEQ ID NO:2, with the exception that the residues are numbered 1-224 in Figures 1A, 1B, and 1C and -37 through 187 in SEQ ID NO:2); "Position": position of the corresponding residue within Figures 1A, 1B, and 1C (which is the identical sequence shown in SEQ ID NO:2, with the exception that the residues are numbered 1-224 in Figures 1A, 1B, and 1C and -37 through 187 in SEQ ID NO:2); I: Alpha, Regions - Garnier-Robson; II: Alpha, Regions - Chou-Fasman; III: Beta, Regions - Garnier-Robson; IV: Beta, Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions - Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot - Kyte-Doolittle; IX: Hydrophobicity Plot - Hopp-Woods; X: Alpha, Amphipathic Regions - Eisenberg; XI: Beta, Amphipathic Regions - Eisenberg; XII: Flexible Regions - Karplus-Schulz; XIII: Antigenic Index - Jameson-Wolf; and XIV: Surface Probability Plot - Emini.

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Detailed Description

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a Ependymin polypeptide having the amino acid sequence shown in SEQ ID NO:2, which was determined by sequencing a cloned cDNA. The nucleotide sequence
5   shown in Figures 1A, 1B, and 1C (SEQ ID NO:1) was obtained by sequencing the HDPIE88 clone, which was deposited on November 14, 1997 at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, and given accession number ATCC 209464. The deposited clone is contained in the pBluescript SK(-) plasmid (Stratagene, La Jolla, CA).

10   The Ependymin protein of the present invention shares sequence homology with the translation products of ependymins from a number of teleost fish including the Zebrafish mRNA for ependymin (Figure 2; SEQ ID NO:6). Zebrafish ependymin, and other ependymins, are thought to be involved in the regulation of extracellular Ca^{2+}-binding. In fact, as the predominant cerebrospinal fluid constituents in many teleost fish, ependymins are believed to be
15   antiadhesive extracellular matrix glycoproteins which function in the processes of cell contact and regeneration.

Nucleic Acid Molecules

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373
20   from Applied Biosystems, Inc., Foster City, CA), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least
25   about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of
30   the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

By "nucleotide sequence" of a nucleic acid molecule or polynucleotide is intended, for a
35   DNA molecule or polynucleotide, a sequence of deoxyribonucleotides, and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U), where each thymidine deoxyribonucleotide (T) in the specified deoxyribonucleotide sequence is replaced by the ribonucleotide uridine (U).

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Using the information provided herein, such as the nucleotide sequence in Figures 1A,
1B, and 1C (SEQ ID NO:1), a nucleic acid molecule of the present invention encoding a
Ependymin polypeptide may be obtained using standard cloning and screening procedures,
such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention,

5   the nucleic acid molecule described in Figures 1A, 1B, and 1C (SEQ ID NO:1) was discovered in
a cDNA library derived from primary dendritic cells.

Additional clones of the same gene were also identified in cDNA libraries from the
following tissues: the KMH2 cell line, placenta, fetal and adult liver, spinal cord, osteoclastoma,
cerebellum, synovial fibroblasts, 12 week old early stage human embryo, adrenal gland tumor,

10   whole brain, Hodgkin's Lymphoma tissue, macrophages, HEL cell line, and chondrosarcoma.

The determined nucleotide sequence of the Ependymin cDNA of Figures 1A, 1B, and
1C (SEQ ID NO:1) contains an open reading frame encoding a protein of 224 amino acid
residues, with an initiation codon at nucleotide positions 296-298 of the nucleotide sequence in
Figures 1A, 1B, and 1C (SEQ ID NO:1), and a deduced molecular weight of about 25.4 kDa.

15   The amino acid sequence of the Ependymin protein shown in SEQ ID NO:2 is about 22.5 %
identical to Zebrafish mRNA for ependymin (Figure 2; Sterrer, S., et al., Neurosci. 37:277-284
(1990); GenBank Accession No. X52502).

As one of ordinary skill would appreciate, due to the possibilities of sequencing errors
discussed above, the actual complete Ependymin polypeptide encoded by the deposited cDNA,

20   which comprises about 224 amino acids, may be somewhat longer or shorter. It will further be
appreciated that, depending on the analytical criteria used for identifying various functional
domains, the exact "address" of the signal peptide and mature regions of the Ependymin
polypeptide may differ slightly from the predicted positions above. For example, the exact
location of the Ependymin signal peptide in SEQ ID NO:2 may vary slightly (e.g., the address

25   may "shift" by about 1 to about 15 residues, more likely about 1 to about 10 residues, and even
more likely about 1 to about 5 residues) depending on the criteria used to define the domain.
In this case, the end of the signal peptide and the beginning of the mature Ependymin molecule
were predicted using the Human Genome Sciences, Inc. (HGSI) SignalP computer algorithm
(Pedersen, A. G. and Nielsen, H. ISMB 5:226-233 (1997)). One of skill in the art will realize

30   that another widely accepted computer algorithm used to predict potential sites of polypeptide
cleavage, PSORT, will predict the cleavage of an N-terminal signal peptide from the Ependymin
polypeptide at a point slightly different from that predicted by the HGSI SignalP algorithm. In
any event, as discussed further below, the invention further provides polypeptides having various
residues deleted from the N-terminus of the complete polypeptide, including polypeptides

35   lacking one or more amino acids from the N-terminus of the mature polypeptide described
herein.

Leader and Mature Sequences

The amino acid sequence of the complete Ependymin protein includes a leader
sequence and a mature protein, as shown in SEQ ID NO:2. More in particular, the present

40   invention provides nucleic acid molecules encoding a mature form of the Ependymin protein.

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Thus, according to the signal hypothesis, once export of the growing protein chain across the rough endoplasmic reticulum has been initiated, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the complete polypeptide to produce a secreted "mature" form of the protein. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature Ependymin polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209464. By the "mature Ependymin polypeptide having the amino acid sequence encoded by the cDNA clone in ATCC Deposit No. 209464" is meant the mature form(s) of the Ependymin protein produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the deposited clone.

In addition, methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the method of McGeoch (*Virus Res.* 3:271-286 (1985)) uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje (*Nucleic Acids Res.* 14:4683-4690 (1986)) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2 where +1 indicates the amino terminus of the mature protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80% (von Heinje, *supra*). However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the deduced amino acid sequence of the complete Ependymin polypeptide was analyzed by the HGSI SignalP computer algorithm (Pedersen, A. G. and Nielsen, H. *ISMB* 5:226-233 (1997)). Using this computer analysis tool, a likely site of signal peptide cleavage was predicted between amino acid residues 37 and 38 of the Ependymin sequence (SEQ ID NO:2). However, the deduced amino acid sequence of the complete Ependymin polypeptide was also analyzed by a computer program designated "PSORT", available from Dr. Kenta Nakai of the Institute for Chemical Research, Kyoto University (Nakai, K. and Kanehisa, M. *Genomics* 14:897-911 (1992)), which is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. Thus, the computation analysis above predicted a single cleavage site within the complete amino acid sequence shown in SEQ ID NO:2.

As one of ordinary skill would appreciate from the above discussions, due to the possibilities of sequencing errors as well as the variability of cleavage sites in different known proteins, the mature Ependymin polypeptide encoded by the deposited cDNA is expected to consist of about 187 amino acids (presumably residues 1 to 187 of SEQ ID NO:2) based on analysis of the Ependymin amino acid sequence using the SignalP computer algorithm, but may

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consist of any number of amino acids in the range of about 187 to 200 amino acids (the mature polypeptide is predicted to be 200 amino acids using the PSORT computer algorithm). Further, the actual leader sequence(s) of this protein is expected to be 37 amino acids (presumably residues -37 through -1 of SEQ ID NO:2) based on analysis of the Ependymin amino acid
5   sequence using the SignalP computer algorithm, but may consist of any number of amino acids in the range of 24-37 amino acids (the signal peptide is predicted to be 24 amino acids using the PSORT computer algorithm).

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA
10   obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA
15   molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present
20   invention further include such molecules produced synthetically.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) with an initiation codon at positions 288-290 of the nucleotide sequence shown in Figures 1A, 1B, and 1C (SEQ ID NO:1).

Also included are DNA molecules comprising the coding sequence for the predicted
25   mature Ependymin protein shown at positions 1-187 of SEQ ID NO:2.

In addition, isolated nucleic acid molecules of the invention include DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the Ependymin protein. Of course, the genetic code and species-specific codon preferences are well known in the art. Thus, it would be routine
30   for one skilled in the art to generate the degenerate variants described above, for instance, to optimize codon expression for a particular host (e.g., change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

In another aspect, the invention provides isolated nucleic acid molecules encoding the Ependymin polypeptide having an amino acid sequence encoded by the cDNA clone contained
35   in the plasmid deposited as ATCC Deposit No. 209464 on November 14, 1997.

Preferably, this nucleic acid molecule will encode the mature polypeptide encoded by the above-described deposited cDNA clone.

The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figures 1A, 1B, and 1C (SEQ ID NO:1) or the nucleotide sequence of the
40   Ependymin cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules,

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particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes, and for detecting expression of the Ependymin gene in human tissue, for instance, by Northern blot analysis.

The present invention is further directed to nucleic acid molecules encoding portions of the nucleotide sequences described herein as well as to fragments of the isolated nucleic acid molecules described herein. In particular, the invention provides a polynucleotide having a nucleotide sequence representing the portion of SEQ ID NO:1 which consists of positions 1-967 of SEQ ID NO:1.

In addition, the invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:1 which have been determined from the following related cDNA clones: HATBS80R (SEQ ID NO:15); HSRAN11R (SEQ ID NO:16); HCECE56R (SEQ ID NO:17); HSNBF20R (SEQ ID NO:18); HPMDJ94R (SEQ ID NO:19); and HE2FK31R (SEQ ID NO:20).

Further, the invention includes a polynucleotide comprising any portion of at least about 30 nucleotides, preferably at least about 50 nucleotides, of SEQ ID NO:1 from residue 1 to 2505.

More generally, by a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNA or the nucleotide sequence shown in Figures 1A, 1B, and 1C (SEQ ID NO:1) is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50-300 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA or as shown in Figures 1A, 1B, and 1C (SEQ ID NO:1). By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNA or the nucleotide sequence as shown in Figures 1A, 1B, and 1C (SEQ ID NO:1). Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding epitope-bearing portions of the Ependymin polypeptide as identified in Figures 3A and 3B and described in more detail below.

Preferred nucleic acid fragments of the present invention also include nucleic acid molecules encoding one or more of the following domains of Human Ependymin: amino acid residues -20 through -10 of SEQ ID NO:2; amino acid residues 1 through 10 of SEQ ID NO:2; amino acid residues 31 through 40 of SEQ ID NO:2; amino acid residues 64 through 70 of SEQ ID NO:2; amino acid residues 76 through 82 of SEQ ID NO:2; amino acid residues 88 through 105 of SEQ ID NO:2; amino acid residues 106 through 117 of SEQ ID NO:2; amino acid residues 129 through 143 of SEQ ID NO:2; amino acid residues 150 through 156 of SEQ ID NO:2; and amino acid residues 162 through 184 of SEQ ID NO:2. These domains are represented as conserved domains CD-I through CD-X in Figures 1A, 1B, and 1C.

In specific embodiments, the polynucleotide fragments of the invention encode antigenic regions. Non-limiting examples of antigenic polypeptides or peptides that can be used to generate Ependymin-specific antibodies include: a polypeptide comprising amino acid

residues from about Ala-1 to about Gln-9 in SEQ ID NO:2; a polypeptide comprising amino
acid residues from about Pro-8 to about Val-16 in SEQ ID NO:2; a polypeptide comprising
amino acid residues from about Gln-19 to about Arg-27 in SEQ ID NO:2; a polypeptide
comprising amino acid residues from about Ile-69 to about Ser-77 in SEQ ID NO:2; a

5   polypeptide comprising amino acid residues from about Asp-86 to about Glu-107 in SEQ ID
NO:2; a polypeptide comprising amino acid residues from about Glu-113 to about Tyr-123 in
SEQ ID NO:2; a polypeptide comprising amino acid residues from about Thr-131 to about
Gln-139 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Leu-159
to about Phe-167 in SEQ ID NO:2; and a polypeptide comprising amino acid residues from

10   about Leu-178 to about Ser-186 in SEQ ID NO:2.

In additional embodiments, the polynucleotides of the invention encode functional
attributes of Human Ependymin. Preferred embodiments of the invention in this regard include
fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"),
beta-sheet and beta-sheet forming regions ("beta-regions"), turn and turn-forming regions

15   ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions,
hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions,
surface-forming regions and high antigenic index regions of Human Ependymin.

The data representing the structural or functional attributes of Human Ependymin set
forth in Figure 4 and/or Table I, as described above, was generated using the various modules

20   and algorithms of the DNA*STAR set on default parameters. In a preferred embodiment, the
data presented in columns VIII, IX, XIII, and XIV of Table I can be used to determine regions
of Human Ependymin which exhibit a high degree of potential for antigenicity. Regions of
high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or IV by
choosing values which represent regions of the polypeptide which are likely to be exposed on

25   the surface of the polypeptide in an environment in which antigen recognition may occur in the
process of initiation of an immune response.

Certain preferred regions in these regards are set out in Figure 4, but may, as shown in
Table I, be represented or identified by using tabular representations of the data presented in
Figure 4. The DNA*STAR computer algorithm used to generate Figure 4 (set on the original

30   default parameters) was used to present the data in Figure 4 in a tabular format (See Table I).
The tabular format of the data in Figure 4 may be used to easily determine specific boundaries
of a preferred region.

The above-mentioned preferred regions set out in Figure 4 and in Table I include, but
are not limited to, regions of the aforementioned types identified by analysis of the amino acid

35   sequence set out in Figures 1A, 1B, and 1C. As set out in Figure 4 and in Table I, such preferred
regions include Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions,
Chou-Fasman alpha-regions, beta-regions, and coil-regions, Kyte-Doolittle hydrophilic regions
and hydrophobic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz
flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic

40   index.

Table I

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Met	1	.	.	B	.	.	T	.	0.31	-0.24	*	*	.	1.07	1.11
Pro	2	.	.	.	.	.	T	C	0.49	-0.17	.	*	.	1.34	0.88
Gly	3	.	.	.	.	T	T	.	0.07	-0.17	*	*	.	1.91	1.06
Arg	4	.	.	B	.	.	T	.	0.57	0.09	*	*	.	0.98	0.89
Ala	5	.	.	B	.	.	.	.	0.64	-0.53	*	*	F	2.20	1.12
Pro	6	.	.	B	B	.	.	.	0.39	-0.47	*	*	F	1.48	1.63
Leu	7	.	.	B	B	.	.	.	0.39	-0.26	*	*	.	0.96	0.62
Arg	8	.	.	B	B	.	.	.	0.39	0.17	*	*	F	0.29	0.95
Thr	9	.	.	B	B	.	.	.	-0.31	0.10	*	*	F	0.07	0.61
Val	10	.	.	B	.	.	T	.	-0.53	0.17	*	*	F	0.25	0.74
Pro	11	.	.	B	.	.	T	.	-0.67	0.17	*	.	F	0.25	0.31
Gly	12	.	.	B	.	.	T	.	-0.44	0.60	*	.	F	-0.05	0.21
Ala	13	.	.	B	.	.	T	.	-0.84	0.61	*	.	.	-0.20	0.29
Leu	14	.	A	B	B	.	.	.	-1.34	0.89	.	.	.	-0.60	0.20
Gly	15	.	A	B	B	.	.	.	-1.30	1.14	.	.	.	-0.60	0.17
Ala	16	.	A	B	B	.	.	.	-1.43	1.40	.	.	.	-0.60	0.14
Trp	17	.	A	B	B	.	.	.	-1.43	1.33	.	.	.	-0.60	0.16
Leu	18	.	A	B	B	.	.	.	-1.66	1.07	.	.	.	-0.60	0.16
Leu	19	.	A	B	B	.	.	.	-1.13	1.33	.	.	.	-0.60	0.13
Gly	20	.	A	.	B	T	.	.	-1.38	1.74	.	.	.	-0.20	0.13
Gly	21	.	.	.	B	.	.	C	-1.08	1.33	.	.	.	-0.40	0.16
Leu	22	.	.	.	B	T	.	.	-1.10	1.56	.	.	.	-0.20	0.21
Trp	23	.	.	.	B	T	.	.	-1.10	1.36	.	.	.	-0.20	0.30
Ala	24	.	.	B	B	.	.	.	-0.96	1.61	.	.	.	-0.60	0.25
Trp	25	.	.	B	B	.	.	.	-0.96	1.76	.	.	.	-0.60	0.16
Thr	26	.	.	B	B	.	.	.	-1.42	1.50	.	.	.	-0.60	0.15
Leu	27	.	.	B	B	.	.	.	-1.28	1.27	.	.	.	-0.60	0.13
Cys	28	.	.	.	B	T	.	.	-1.29	1.34	.	.	.	-0.20	0.06
Gly	29	.	.	.	B	T	.	.	-1.51	0.81	.	.	.	-0.20	0.06
Leu	30	.	.	.	B	T	.	.	-1.57	1.01	.	.	.	-0.20	0.06
Cys	31	.	.	B	.	.	T	.	-1.84	0.76	.	.	.	-0.20	0.11
Ser	32	.	.	B	.	.	T	.	-1.89	0.69	.	.	.	-0.20	0.11
Leu	33	.	.	B	.	.	T	.	-1.57	0.90	.	.	.	-0.20	0.10
Gly	34	.	.	B	.	.	T	.	-1.81	0.64	.	.	.	-0.20	0.19
Ala	35	.	.	B	B	.	.	.	-1.21	0.57	.	.	.	-0.60	0.14
Val	36	.	.	B	B	.	.	.	-0.43	0.61	.	.	.	-0.60	0.26
Gly	37	.	.	B	B	.	.	.	-0.34	-0.07	.	.	.	0.58	0.52
Ala	38	.	.	B	.	.	.	.	-0.20	-0.07	.	.	F	1.21	0.80
Pro	39	.	.	B	.	.	.	.	0.14	-0.00	*	.	F	1.49	0.58
Arg	40	.	.	.	.	.	T	C	0.14	-0.24	*	.	F	2.32	1.01
Pro	41	.	.	.	.	T	T	.	0.79	-0.17	.	.	F	2.80	1.01
Cys	42	.	.	.	.	T	T	.	1.13	-0.24	*	.	F	2.52	1.01
Gln	43	.	.	B	.	.	T	.	1.72	-0.27	*	.	F	1.69	0.89
Ala	44	.	.	B	.	.	.	.	1.64	0.13	*	*	F	0.61	1.00
Pro	45	.	.	.	.	.	.	C	1.53	0.61	*	*	F	0.58	1.96
Gln	46	.	.	.	.	T	.	.	1.40	0.04	*	*	F	1.00	1.96
Gln	47	.	.	.	.	T	.	.	2.18	0.07	.	*	F	1.20	1.92
Trp	48	.	.	.	.	T	.	.	2.18	-0.43	.	*	F	2.00	2.43
Glu	49	.	.	.	.	.	.	C	1.91	-0.46	.	*	F	2.00	2.43
Gly	50	.	.	.	B	T	.	.	1.52	-0.21	.	*	F	1.80	1.04
Arg	51	.	.	.	B	T	.	.	1.28	-0.00	.	*	F	1.45	0.98
Gln	52	.	.	B	B	.	.	.	1.28	-0.16	.	*	.	0.70	0.89
Val	53	.	.	B	B	.	.	.	1.57	0.24	.	.	.	0.05	1.55
Met	54	.	.	B	B	.	.	.	1.27	0.21	.	.	.	-0.15	1.37
Tyr	55	.	.	B	B	.	.	.	1.31	0.60	*	.	.	-0.45	1.06
Gln	56	.	.	B	B	.	.	.	0.86	0.59	*	.	F	0.04	1.91

Table I (continued)

	Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
5	Gln	57	.	.	B	B	.	.	.	0.97	0.37	.	.	F	0.68	1.91
	Ser	58	.	.	.	.	.	T	C	1.82	-0.24	.	.	F	2.22	2.39
	Ser	59	.	.	.	.	.	T	C	2.12	-0.60	*	.	F	2.86	2.22
	Gly	60	.	.	.	.	T	T	.	2.48	-0.61	*	.	F	3.40	1.72
	Arg	61	.	.	.	.	T	T	.	1.89	-1.01	*	.	F	3.06	2.51
10	Asn	62	.	.	.	.	.	T	C	1.08	-0.90	*	.	F	2.52	1.89
	Ser	63	.	.	B	.	.	T	.	0.57	-0.60	*	.	F	1.98	1.58
	Arg	64	.	.	B	.	.	T	.	0.57	-0.34	*	.	F	1.19	0.66
	Ala	65	.	.	B	.	.	T	.	0.67	0.04	*	*	.	0.10	0.55
	Leu	66	.	.	B	.	.	.	.	0.56	0.40	*	*	.	-0.10	0.65
15	Leu	67	.	.	B	.	.	.	.	0.21	0.01	.	*	.	-0.10	0.55
	Ser	68	.	.	B	.	.	T	.	-0.30	0.44	.	*	.	-0.20	0.54
	Tyr	69	.	.	B	.	.	T	.	-0.41	0.63	.	*	.	-0.20	0.54
	Asp	70	.	.	.	.	T	T	.	0.18	0.34	.	.	F	0.80	1.06
	Gly	71	.	.	.	.	T	T	.	1.10	0.06	.	*	F	0.80	1.36
20	Leu	72	.	.	.	.	.	.	C	1.06	-0.33	*	*	F	1.00	1.70
	Asn	73	.	.	B	B	.	.	.	1.47	-0.44	*	*	F	0.45	0.76
	Gln	74	.	.	B	B	.	.	.	0.86	-0.44	*	*	F	0.60	1.50
	Arg	75	.	.	B	B	.	.	.	0.04	-0.23	*	*	F	0.60	1.35
	Val	76	.	A	B	B	.	.	.	0.39	-0.23	*	*	.	0.30	0.69
25	Arg	77	.	A	B	B	.	.	.	1.20	-0.63	*	*	.	0.60	0.67
	Val	78	.	A	B	B	.	.	.	1.31	-1.03	.	*	.	0.60	0.59
	Leu	79	.	A	B	B	.	.	.	1.36	-1.03	.	*	.	0.75	1.56
	Asp	80	.	A	B	B	.	.	.	0.66	-1.67	.	*	F	0.90	1.59
	Glu	81	.	A	B	.	T	.	.	0.70	-1.17	*	.	F	1.30	2.16
30	Arg	82	.	A	.	.	T	.	.	-0.30	-1.13	*	.	F	1.30	2.16
	Lys	83	.	A	.	.	T	.	.	0.34	-1.13	.	.	F	1.15	0.91
	Ala	84	.	A	.	.	T	.	.	0.49	-0.70	.	*	.	1.00	0.81
	Leu	85	.	A	B	.	.	.	.	0.53	-0.13	*	.	.	0.30	0.22
	Ile	86	.	.	B	.	.	T	.	0.64	-0.13	*	.	.	0.70	0.22
35	Pro	87	.	.	B	.	.	T	.	-0.28	-0.13	*	.	.	0.70	0.43
	Cys	88	.	.	B	.	.	T	.	-1.02	0.06	*	.	.	0.10	0.43
	Lys	89	.	.	B	.	.	T	.	-0.43	0.16	*	.	.	0.10	0.53
	Arg	90	.	.	B	.	.	.	.	0.13	-0.53	*	.	.	0.80	0.60
	Leu	91	.	.	B	B	.	.	.	0.13	-0.20	*	.	.	0.45	1.74
40	Phe	92	.	.	B	B	.	.	.	-0.47	-0.09	*	.	.	0.30	0.61
	Glu	93	.	.	B	B	.	.	.	-0.61	0.60	*	.	.	-0.60	0.26
	Tyr	94	.	.	B	B	.	.	.	-0.90	1.29	*	.	.	-0.60	0.46
	Ile	95	.	.	B	B	.	.	.	-0.97	1.36	*	.	.	-0.60	0.54
	Leu	96	.	.	B	B	.	.	.	-0.16	0.57	.	.	.	-0.60	0.57
45	Leu	97	.	.	B	B	.	.	.	0.20	0.57	.	.	.	0.50	0.81
	Tyr	98	.	.	.	.	T	T	.	-0.66	0.24	.	.	.	0.50	0.73
	Lys	99	.	.	.	.	T	T	.	-1.01	0.20	.	.	.	0.50	0.87
	Asp	100	.	.	.	.	T	T	.	-0.82	0.13	.	.	.	0.10	0.48
	Gly	101	.	.	B	.	.	T	.	-0.01	0.23	.	*	.	0.30	0.42
50	Val	102	.	.	B	B	.	.	.	-0.09	-0.13	.	*	.	-0.60	0.18
	Met	103	.	.	B	B	.	.	.	0.16	0.56	.	*	.	-0.60	0.30
	Phe	104	.	.	B	B	.	.	.	0.11	0.56	.	*	.	-0.30	0.69
	Gln	105	.	.	B	B	.	.	.	-0.48	0.53	*	.	.	0.30	0.70
	Ile	106	.	.	B	B	.	.	.	-0.44	0.39	*	*	F	0.90	1.17
55	Asp	107	.	.	B	B	.	.	.	0.46	0.26	*	*	F	2.70	1.36
	Gln	108	.	.	.	.	T	.	.	1.06	-0.53	*	*	F	3.00	3.35
	Ala	109	.	.	.	.	T	.	.	1.09	-0.53	*	*	F	2.70	1.07
	Thr	110	.	.	.	.	T	.	.	0.79	-0.64	*	.	F	1.95	0.83
	Lys	111	.	.	.	.	T	.	.	1.72	-0.26	*	.			
60																

Table I (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Gln	112	.	.	.	.	T	.	.	1.12	-0.66	*	.	F	2.10	1.65
Cys	113	.	.	.	.	T	.	.	0.81	-0.54	*	*	F	1.80	1.13
Ser	114	.	.	B	.	.	.	.	0.59	-0.54	*	*	F	0.95	0.81
Lys	115	.	.	B	.	.	.	.	0.59	0.14	.	.	F	0.05	0.39
Met	116	.	.	B	.	.	.	.	0.54	0.23	.	.	.	0.05	1.04
Thr	117	.	.	B	.	.	.	.	0.33	0.06	.	.	.	0.05	1.35
Leu	118	.	.	B	.	.	.	.	0.71	0.10	.	*	F	0.20	1.04
Thr	119	.	.	B	.	.	.	.	1.01	1.01	.	*	F	-0.10	1.11
Gln	120	.	.	B	.	.	.	.	0.76	0.40	*	*	F	0.20	1.28
Pro	121	.	.	.	.	T	.	.	0.54	0.34	*	.	F	0.60	2.41
Trp	122	.	.	.	.	T	.	.	0.86	0.34	*	.	F	0.60	1.37
Asp	123	.	.	.	.	.	T	C	0.78	-0.14	*	.	F	1.20	1.33
Pro	124	.	.	B	.	.	T	.	0.88	0.14	.	.	F	0.25	0.60
Leu	125	.	.	.	.	T	T	.	0.88	0.14	.	.	F	0.93	0.88
Asp	126	.	.	B	.	.	T	.	1.09	-0.37	.	.	F	1.41	0.92
Ile	127	.	.	.	.	.	.	C	1.08	0.03	.	.	F	1.09	0.95
Pro	128	.	.	.	.	.	T	C	0.77	-0.01	.	*	F	2.32	1.55
Gln	129	.	.	.	.	T	T	.	0.28	-0.21	.	*	F	2.80	1.34
Asn	130	.	.	.	.	.	T	C	1.09	0.57	.	*	F	1.42	1.65
Ser	131	.	.	.	.	.	T	C	1.09	-0.11	.	*	F	2.04	1.85
Thr	132	.	.	B	.	.	.	.	1.98	-0.54	.	.	F	1.66	1.79
Phe	133	.	.	B	.	.	.	.	1.94	-0.54	.	.	F	1.60	1.92
Glu	134	.	.	B	.	.	.	.	1.64	-0.19	.	*	F	1.24	2.25
Asp	135	.	.	B	.	.	T	.	0.76	-0.19	.	*	F	1.66	2.09
Gln	136	.	.	B	.	.	T	.	0.71	0.01	.	*	.	1.13	1.69
Tyr	137	.	.	.	.	T	T	.	0.68	-0.34	.	*	.	2.20	0.97
Ser	138	.	.	.	.	T	T	.	1.17	0.09	.	.	.	1.38	0.57
Ile	139	.	.	.	.	T	.	.	1.17	0.51	.	.	F	0.97	0.51
Gly	140	.	.	.	.	.	.	C	1.17	0.51	.	.	F	0.71	0.56
Gly	141	.	.	.	.	.	.	C	1.17	-0.24	.	*	F	1.55	0.73
Pro	142	.	.	.	.	.	.	C	0.52	-0.23	.	.	F	1.64	1.80
Gln	143	.	.	.	B	.	.	C	0.51	-0.23	.	*	F	1.60	1.28
Glu	144	.	.	B	B	.	.	.	0.54	-0.17	.	*	F	1.24	1.86
Gln	145	.	.	B	B	.	.	.	0.89	0.04	.	*	F	0.33	0.89
Ile	146	.	.	B	B	.	.	.	1.23	0.01	.	*	F	0.17	0.89
Thr	147	.	.	B	B	.	.	.	1.16	-0.39	.	*	.	0.46	0.89
Val	148	.	.	B	B	.	.	.	0.86	0.53	.	*	.	-0.60	0.54
Gln	149	.	.	B	B	.	.	.	0.86	0.51	.	*	.	-0.11	1.04
Glu	150	.	.	B	B	.	.	.	0.97	-0.17	.	*	F	1.28	1.20
Trp	151	.	.	.	.	T	T	.	1.90	-0.66	.	.	F	2.72	3.17
Ser	152	.	.	.	.	.	T	C	1.91	-1.30	.	.	F	2.86	3.66
Asp	153	.	.	.	.	T	T	.	2.18	-1.31	*	*	F	3.40	2.83
Arg	154	.	.	.	.	T	T	.	2.29	-0.81	*	*	F	3.06	2.72
Lys	155	.	.	.	.	T	.	.	1.99	-1.73	*	*	F	2.72	3.98
Ser	156	.	.	.	.	.	.	C	2.03	-1.73	*	*	F	2.38	3.19
Ala	157	.	.	.	.	.	T	C	2.33	-0.97	*	.	F	2.44	2.55
Arg	158	.	.	.	.	.	T	C	2.02	-0.97	*	*	F	2.30	2.21
Ser	159	.	.	B	.	.	T	.	1.62	-0.49	*	*	F	2.00	2.38
Tyr	160	.	.	B	.	.	T	.	0.69	0.04	*	.	F	1.20	2.48
Glu	161	.	.	B	B	.	.	.	0.64	0.23	*	*	F	0.45	0.89
Thr	162	.	.	B	B	.	.	.	0.34	0.66	*	.	.	-0.20	0.65
Trp	163	.	.	B	B	.	.	.	-0.01	0.96	*	.	.	-0.40	0.29
Ile	164	.	.	B	B	.	.	.	-0.02	0.96	.	.	.	-0.60	0.27
Gly	165	.	.	B	B	.	.	.	-0.63	1.44	.	.	.	-0.60	0.19
Ile	166	.	.	B	B	.	.	.	-0.59	1.60	.	.	.	-0.60	0.53
Tyr	167	.	.	B	B	.	.	.	-0.28	0.69	.	.	.	-0.60	0.53

Table I (continued)

	Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
5	Thr	168	.	.	B	B	.	.	.	-0.66	-0.00	.	.	.	0.30	0.90
	Val	169	.	.	.	B	T	.	.	-0.01	0.14	.	.	.	0.24	0.69
	Lys	170	.	.	.	.	T	T	.	0.12	0.21	*	.	F	0.93	0.69
	Asp	171	.	.	.	.	T	T	.	0.16	-0.11	.	*	.	1.52	0.74
	Cys	172	.	.	B	.	.	T	.	0.40	0.04	.	*	.	0.66	0.74
10	Tyr	173	.	.	B	.	.	T	.	0.71	-0.20	*	.	.	1.40	0.64
	Pro	174	.	.	B	.	.	.	.	1.26	-0.20	*	*	.	1.06	0.66
	Val	175	.	.	B	B	.	.	.	0.51	0.29	*	.	.	0.27	1.78
	Gln	176	.	.	B	B	.	.	.	0.20	0.50	.	*	F	-0.17	0.99
	Glu	177	.	.	B	B	.	.	.	-0.02	0.23	.	*	F	-0.01	0.92
15	Thr	178	.	.	B	B	.	.	.	0.22	0.49	.	*	F	-0.45	0.87
	Phe	179	.	.	B	B	.	.	.	0.19	0.24	.	*	.	-0.30	0.81
	Thr	180	.	.	B	B	.	.	.	0.74	0.60	.	*	.	-0.60	0.73
	Ile	181	.	.	B	B	.	.	.	-0.11	0.99	.	*	.	-0.60	0.68
	Asn	182	.	.	B	B	.	.	.	-1.00	1.14	.	*	.	-0.60	0.58
20	Tyr	183	.	.	B	B	.	.	.	-1.50	1.04	.	*	.	-0.60	0.28
	Ser	184	.	.	B	B	.	.	.	-1.10	1.24	.	*	.	-0.60	0.33
	Val	185	.	.	B	B	.	.	.	-1.10	0.94	*	*	.	-0.60	0.28
	Ile	186	.	.	B	B	.	.	.	-0.10	1.03	*	*	.	-0.60	0.25
	Leu	187	.	.	B	B	.	.	.	-0.80	0.27	*	*	.	-0.30	0.37
25	Ser	188	.	.	B	B	.	.	.	-1.26	0.67	*	*	.	-0.60	0.43
	Thr	189	.	.	B	B	.	.	.	-0.96	0.81	*	*	F	-0.45	0.54
	Arg	190	.	.	B	B	.	.	.	-0.99	0.13	.	*	.	-0.15	1.09
	Phe	191	.	.	B	B	.	.	.	-0.10	0.13	.	*	.	-0.30	0.57
	Phe	192	.	.	B	B	.	.	.	-0.10	0.14	.	*	.	-0.30	0.68
30	Asp	193	.	.	B	B	.	.	.	-0.14	0.34	*	*	.	-0.30	0.29
	Ile	194	.	.	B	B	.	.	.	-0.72	0.77	.	*	.	-0.60	0.33
	Gln	195	.	.	B	B	.	.	.	-0.79	0.67	*	*	.	-0.60	0.27
	Leu	196	.	.	B	B	.	.	.	-0.09	-0.11	*	*	.	0.51	0.32
	Gly	197	.	.	.	.	T	.	.	0.40	-0.11	.	*	.	1.32	0.76
35	Ile	198	.	.	.	.	T	.	.	0.10	-0.37	.	*	.	1.53	0.68
	Lys	199	.	.	.	.	.	.	C	0.13	-0.39	*	*	F	1.84	1.10
	Asp	200	.	.	.	.	.	T	C	-0.57	-0.43	.	*	F	2.10	0.83
	Pro	201	.	.	B	.	.	T	.	-0.07	-0.07	.	.	F	1.84	1.02
	Ser	202	.	.	B	.	.	T	.	0.07	-0.27	.	.	F	1.48	0.74
40	Val	203	.	.	B	.	.	T	.	0.74	0.16	.	.	.	0.52	0.68
	Phe	204	.	.	B	.	.	.	.	0.40	0.59	.	.	.	-0.19	0.68
	Thr	205	.	.	.	.	.	.	C	0.09	0.54	.	.	F	-0.05	0.68
	Pro	206	.	.	.	.	.	T	C	-0.37	0.64	.	.	F	0.30	1.32
	Pro	207	.	.	.	.	T	T	.	-0.07	0.57	.	.	F	0.35	0.82
45	Ser	208	.	.	.	.	T	T	.	0.19	0.19	.	.	F	0.65	0.98
	Thr	209	.	.	.	.	T	T	.	0.30	0.31	.	.	F	0.65	0.63
	Cys	210	.	A	B	.	.	.	.	0.61	0.39	.	.	.	-0.30	0.41
	Gln	211	.	A	B	.	.	.	.	0.01	0.36	.	.	.	-0.30	0.53
	Met	212	.	A	B	.	.	.	.	0.22	0.66	.	.	.	-0.60	0.30
50	Ala	213	.	A	B	.	.	.	.	0.57	0.17	.	.	.	-0.30	0.98
	Gln	214	A	A	.	.	.	.	.	0.28	-0.40	.	.	.	0.45	1.13
	Leu	215	A	A	.	.	.	.	.	0.64	-0.19	.	.	.	0.76	1.13
	Glu	216	.	A	B	.	.	.	.	0.64	-0.41	*	.	.	1.07	1.50
	Lys	217	.	A	B	.	.	.	.	1.24	-0.91	*	.	F	1.83	1.50
55	Met	218	.	A	.	.	T	.	.	1.17	-1.31	*	.	F	2.54	3.05
	Ser	219	.	.	.	.	T	T	.	0.87	-1.43	*	.	F	3.10	0.94
	Glu	220	.	.	.	.	T	T	.	1.39	-1.04	*	.	F	2.79	0.63
	Asp	221	.	.	.	.	T	T	.	1.00	-0.13	.	*	F	2.18	0.67
	Cys	222	.	.	.	.	T	T	.	0.57	-0.31	.	*	.	1.72	0.64
60	Ser	223	.	.	.	.	T	.	.	0.78	-0.27	.	.	.	1.21	0.47
	Trp	224	.	.	.	.	T	.	.	0.69	0.16	.	.	.	0.30	0.36

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Among highly preferred fragments in this regard are those that comprise regions of Human Ependymin that combine several structural features, such as several of the features set out above.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the cDNA clone contained in ATCC Deposit No. 209464. By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 (e.g., 50) nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in Figures 1A, 1B, and 1C (SEQ ID NO:1)). Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of the Ependymin cDNA shown in Figures 1A, 1B, and 1C (SEQ ID NO:1)), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

As indicated, nucleic acid molecules of the present invention which encode a Ependymin polypeptide may include, but are not limited to those encoding the amino acid sequence of the mature polypeptide, by itself; and the coding sequence for the mature polypeptide and additional sequences, such as those encoding the about 37 amino acid leader or secretory sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences.

Also encoded by nucleic acids of the invention are the above protein sequences together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities.

Thus, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton

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Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described by Gentz and colleagues (*Proc. Natl. Acad. Sci. USA* 86:821-824 (1989)), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the

5   influenza hemagglutinin protein, which has been described by Wilson and coworkers (*Cell* 37:767 (1984)). As discussed below, other such fusion proteins include the Ependymin fused to Fc at the N- or C-terminus.

Variant and Mutant Polynucleotides

The present invention further relates to variants of the nucleic acid molecules of the

10   present invention, which encode portions, analogs or derivatives of the Ependymin protein. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (*Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985)). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

15   Such variants include those produced by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not

20   alter the properties and activities of the Ependymin protein or portions thereof. Also especially preferred in this regard are conservative substitutions.

Most highly preferred are nucleic acid molecules encoding the mature protein having the amino acid sequence shown in SEQ ID NO:2 or the mature Ependymin amino acid sequence encoded by the deposited cDNA clone.

25   Thus, one aspect of the invention provides an isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding the Ependymin polypeptide having the complete amino acid sequence in SEQ ID NO:2 (i.e., positions -37 to 187 of SEQ ID NO:2); (b) a nucleotide sequence encoding the Ependymin polypeptide having the complete amino acid sequence in

30   SEQ ID NO:2 excepting the N-terminal methionine (i.e., positions -36 to 187 of SEQ ID NO:2); (c) a nucleotide sequence encoding the predicted mature Ependymin polypeptide having the amino acid sequence at positions 1 to 187 in SEQ ID NO:2; (d) a nucleotide sequence encoding the Ependymin polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209464; (e) a nucleotide sequence encoding the

35   Ependymin polypeptide having the complete amino acid sequence excepting the N-terminal methionine encoded by the cDNA clone contained in ATCC Deposit No. 209464; (f) a nucleotide sequence encoding the mature Ependymin polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209464; and (g) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e) or

40   (f), above.

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Further embodiments of the invention include isolated nucleic acid molecules that comprise a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f) or (g), above, or a polynucleotide which hybridizes under stringent

5   hybridization conditions to a polynucleotide in (a), (b), (c), (d), (e), (f) or (g), above. This polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues. An additional nucleic acid embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an

10 epitope-bearing portion of a Ependymin polypeptide having an amino acid sequence in (a), (b), (c), (d), (e) or (f), above. A further nucleic acid embodiment of the invention relates to an isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of a Ependymin polypeptide having an amino acid sequence which contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid

15 substitutions, even more preferably, not more than 40 conservative amino acid substitutions, still more preferably, not more than 30 conservative amino acid substitutions, and still even more preferably, not more than 20 conservative amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a polynucleotide which encodes the amino acid sequence of a Ependymin polypeptide to have an amino acid sequence which contains not

20 more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of Ependymin polypeptides or peptides by recombinant techniques.

25 By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a Ependymin polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the Ependymin polypeptide. In other words, to obtain

30 a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide

35 sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in Figures 1A,

40 1B, and 1C, or to the nucleotides sequence of the deposited cDNA clone HDPIE88 can be determined conventionally using known computer programs such as the Bestfit program

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(Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). Bestfit uses the local homology algorithm of Smith and Waterman to find the best segment of homology between two sequences (*Advances in Applied Mathematics* 2:482-489 (1981)). When using Bestfit or any

5   other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed. A preferred method for determining the best overall

10   match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag and colleagues (*Comp. App. Biosci.* 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global

15   sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

20   If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number

25   of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is

30   used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to

35   determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were

40   perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are

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internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual

5   corrections are to made for the purposes of the present invention.

The present application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figures 1A, 1B, and 1C (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNA, irrespective of whether they encode a polypeptide having Ependymin activity. This is because even where a particular

10   nucleic acid molecule does not encode a polypeptide having Ependymin activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having Ependymin activity include, *inter alia*, (1) isolating the Ependymin gene or allelic variants thereof in a cDNA library; (2) *in situ*

15   hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the Ependymin gene, as described by Verma and colleagues (*Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988)); and Northern Blot analysis for detecting Ependymin mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 90%, 95%, 96%,

20   97%, 98% or 99% identical to the nucleic acid sequence shown in Figures 1A, 1B, and 1C (SEQ ID NO:1) or to the nucleic acid sequence of the deposited cDNA which do, in fact, encode a polypeptide having Ependymin protein activity. By "a polypeptide having Ependymin activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the mature Ependymin protein of the invention, as measured in a particular biological assay.

25   For example, the Ependymin protein of the present invention binds Ca^{2+} to a high degree. Maruyama and colleagues (*J. Biochem.* **95**:511-519 (1984)), Ganss and Hoffman (*Eur. J. Biochem.* **217**:275-280 (1993)), and Schmidt and Makiola (*Neuro. Chem. (Life Sci. Adv.)* **10**:161-171 (1991)) each demonstrate a convenient laboratory method to determine and roughly quantitate the amount of radiolabeled Ca^{2+} which a given polypeptide, such as

30   Ependymin of the present invention, can bind. Briefly, 5-10 μg protein is used for each analysis. The protein samples are separated by conventional SDS/PAGE using a 13% polyacrylamide gel according to methods which are well-known to one of ordinary skill in the art. Following SDS/PAGE, the proteins are transferred to a nitrocellulose filter also according to methods which are well-known to one of ordinary skill in the art (for example, such a method is

35   provided by Towbin, H., *et al.*, *Proc. Natl. Acad. Sci. USA* **76**:4350-4354 (1979)). After the protein is transferred to the nitrocellulose filter, the blot is hybridized with 200 ml $^{45}CaCl_2$ (300 μCi) in the presence of 5 mM $MgCl_2$. To reduce non-specific binding of Ca^{2+}, the transfer buffer is washed out by three changes (20 minutes each) of 10 mM imidazole/HCl buffer, pH 6.8, containing 5 mM $MgCl_2$ and 60 mM KCl. In such analyses, it is convenient to use a

40   well-known, strong Ca^{2+}-binding protein, such as calmodulin (Pharmacia) and a well-known, protein which does not bind Ca^{2+}, such as bovine serum albumin (Sigma) as controls. To

quantitate the amount of Ca^{2+} bound to a specific protein, the region of the nitrocellulose filter which to which the protein is bound is cut out and placed in a standard scintillation vial. The vial is then filled with 4 ml scintillation cocktail (for example, Quickszint 361; Zinsser, Maidenhead, U. K.) and counted in a liquid scintillation counter (for example, the Wallac 1410,

5   Pharmacia). Using such an analysis, one of ordinary skill in the art may easily ascertain useful qualitative and quantitative information regarding proteins such as Ependymin, or muteins thereof, of the present invention such as the amount of Ca^{2+} that the protein will bind and the apparent molecular mass of the protein. Ca^{2+}-binding activity is a useful indicator of the potential for more complex biological activities.

10   Ependymin protein binds Ca^{2+} in a dose-dependent manner in the above-described assay. Thus, "a polypeptide having Ependymin protein activity" includes polypeptides that also exhibit any of the same Ca^{2+}-binding activities in the above-described assays in a dose-dependent manner. Although the degree of dose-dependent activity need not be identical to that of the Ependymin protein, preferably, "a polypeptide having Ependymin protein activity" will exhibit

15   substantially similar dose-dependence in a given activity as compared to the Ependymin protein (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity relative to the reference Ependymin protein).

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will

20   immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNA or the nucleic acid sequence shown in Figures 1A, 1B, and 1C (SEQ ID NO:1) will encode a polypeptide "having Ependymin protein activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled

25   artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having Ependymin protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a

30   second aliphatic amino acid), as further described below.

Vectors and Host Cells

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of Ependymin polypeptides or fragments thereof by recombinant

35   techniques. The vector may be, for example, a phage, plasmid, viral or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a

40   calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it

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may be packaged *in vitro* using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac, trp, phoA* and *tac* promoters, the SV40 early and late
5 promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately
10 positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not
15 limited to, bacterial cells, such as *E. coli, Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293 and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Vectors preferred for use in bacteria include pHE4-5 (ATCC Accession No. 209311;
20 and variations thereof), pQE70, pQE60 and pQE-9 (QIAGEN, Inc., *supra*); pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1, and pSG (Stratagene); and pSVK3, pBPV, pMSG and pSVL (Pharmacia). Other suitable vectors will be readily apparent to the skilled
25 artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals (for example, Davis, *et al., Basic Methods In Molecular Biology*
30 (1986)).

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during
35 purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous
40 region from immunoglobulin that is useful to stabilize and purify proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various

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portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able
5   to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of
10   hIL-5 (Bennett, D., et al., J. Molecular Recognition **8**:52-58 (1995); Johanson, K., et al., J. Biol. Chem. **270**:9459-9471 (1995)).

The Ependymin protein can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic
15   interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant
20   techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is
25   well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

30   *Polypeptides and Fragments*

The invention further provides an isolated Ependymin polypeptide having the amino acid sequence encoded by the deposited cDNA, or the amino acid sequence in SEQ ID NO:2, or a peptide or polypeptide comprising a portion of the above polypeptides.

Variant and Mutant Polypeptides

35   To improve or alter the characteristics of Ependymin polypeptides, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better

solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions.

N-Terminal and C-Terminal Deletion Mutants

For instance, for many proteins, including the extracellular domain of a membrane
5   associated protein or the mature form(s) of a secreted protein, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. For instance, Ron and colleagues (*J. Biol. Chem.*, **268**:2984-2988 (1993)) reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 N-terminal amino acid residues were missing. In the present case, since the protein of the invention is a
10   member of the ependymin polypeptide family, deletions of N-terminal amino acids up to the cysteine at position 5 of SEQ ID NO:2 may retain some biological activity such as Ca^{2+}-binding or the ability to modulate regeneration. Polypeptides having further N-terminal deletions including the Cys-5 residue in SEQ ID NO:2 would not be expected to retain such biological activities because this residue is conserved in each of the ependymin-related polypeptides shown
15   in Figures 3A and 3B and it is likely that a cysteine in such a position may be required for forming a disulfide bridge to provide structural stability which is needed for receptor binding and signal transduction.

However, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other
20   biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature of the protein generally will be retained when less than the majority of the residues of the complete or mature protein are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine
25   methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the Ependymin shown in SEQ ID NO:2, up to the cysteine residue at position number 5, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the
30   amino acid sequence of residues n^1-187 of SEQ ID NO:2, where n^1 is an integer in the range of -37 to 4, and 5 is the position of the first residue from the N-terminus of the complete Ependymin polypeptide (shown in SEQ ID NO:2) believed to be required for the Ca^{2+}-binding or the regeneration modulatory activities of the Ependymin protein.

More in particular, the invention provides polynucleotides encoding polypeptides having
35   the amino acid sequence of residues of -37-187, -36-187, -35-187, -34-187, -33-187, -32-187, -31-187, -30-187, -29-187, -28-187, -27-187, -26-187, -25-187, -24-187, -23-187, -22-187, -21-187, -20-187, -19-187, -18-187, -17-187, -16-187, -15-187, -14-187, -13-187, -12-187, -11-187, -10-187, -9-187, -8-187, -7-187, -6-187, -5-187, -4-187, -3-187, -2-187, -1-187, 1-187, 2-187, 3-187, 4-187, and 5-187 of SEQ ID NO:2. Polynucleotides encoding these
40   polypeptides also are provided.

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Similarly, many examples of biologically functional C-terminal deletion muteins are
known. For instance, Interferon gamma shows up to ten times higher activities by deleting 8-10
amino acid residues from the carboxy terminus of the protein (Dobeli, *et al., J. Biotechnology*
7:199-216 (1988)). In the present case, since the protein of the invention is a member of the

5   ependymin polypeptide family, deletions of C-terminal amino acids up to the cysteine at
position 173 of SEQ ID NO:2 may retain some biological activity such as Ca^{2+}-binding or the
ability to modulate regeneration. Polypeptides having further C-terminal deletions including the
cysteine residue at position 173 of SEQ ID NO:2 would not be expected to retain such
biological activities because this residue is conserved in each of the ependymin-related

10   polypeptides shown in Figures 3A and 3B and it is likely that a cysteine in such a position may
be required for forming a disulfide bridge to provide structural stability which is needed for
receptor binding and signal transduction.

However, even if deletion of one or more amino acids from the C-terminus of a protein
results in modification of loss of one or more biological functions of the protein, other

15   biological activities may still be retained. Thus, the ability of the shortened protein to induce
and/or bind to antibodies which recognize the complete or mature of the protein generally will
be retained when less than the majority of the residues of the complete or mature protein are
removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of
a complete protein retains such immunologic activities can readily be determined by routine

20   methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more
residues from the carboxy terminus of the amino acid sequence of the Ependymin shown in
SEQ ID NO:2, up to the cysteine residue at position 173 of SEQ ID NO:2, and polynucleotides
encoding such polypeptides. In particular, the present invention provides polypeptides having

25   the amino acid sequence of residues -37-m^1 of the amino acid sequence in SEQ ID NO:2, where
m^1 is any integer in the range of 173 to 187, and residue 173 is the position of the first residue
from the C- terminus of the complete Ependymin polypeptide (shown in SEQ ID NO:2)
believed to be required for the Ca^{2+}-binding or the regeneration modulatory abilities of the
Ependymin protein.

30   More in particular, the invention provides polynucleotides encoding polypeptides having
the amino acid sequence of residues -37-173, -37-174, -37-175, -37-176, -37-177, -37-178,
-37-179, -37-180, -37-181, -37-182, -37-183, -37-184, -37-185, -37-186, and -37-187 of SEQ
ID NO:2. Polynucleotides encoding these polypeptides also are provided.

The invention also provides polypeptides having one or more amino acids deleted from

35   both the amino and the carboxyl termini, which may be described generally as having residues
n^1-m^1 of SEQ ID NO:2, where n^1 and m^1 are integers as described above.

Also included are a nucleotide sequence encoding a polypeptide consisting of a portion
of the complete Ependymin amino acid sequence encoded by the cDNA clone contained in
ATCC Deposit No. 209464, where this portion excludes from 1 to about 42 amino acids from

40   the amino terminus of the complete amino acid sequence encoded by the cDNA clone contained
in ATCC Deposit No. 209464, or from 1 to about 15 amino acids from the carboxy terminus, or

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any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209464. Polynucleotides encoding all of the above deletion mutant polypeptide forms also are provided.

As mentioned above, even if deletion of one or more amino acids from the N-terminus of a protein results in modification of loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened Human Ependymin mutein to induce and/or bind to antibodies which recognize the complete or mature of the protein generally will be retained when less than the majority of the residues of the complete or mature protein are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a Human Ependymin mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immungenic activities. In fact, peptides composed of as few as six Human Ependymin amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the Human Ependymin amino acid sequence shown in SEQ ID NO:2, up to the serine acid residue at position number 219 and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues n^2-224 of Figures 1A, 1B, and 1C (SEQ ID NO:2), where n^2 is an integer in the range of 2 to 219, and 220 is the position of the first residue from the N-terminus of the complete Human Ependymin polypeptide believed to be required for at least immunogenic activity of the Human Ependymin protein.

More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of P-2 to W-224; G-3 to W-224; R-4 to W-224; A-5 to W-224; P-6 to W-224; L-7 to W-224; R-8 to W-224; T-9 to W-224; V-10 to W-224; P-11 to W-224; G-12 to W-224; A-13 to W-224; L-14 to W-224; G-15 to W-224; A-16 to W-224; W-17 to W-224; L-18 to W-224; L-19 to W-224; G-20 to W-224; G-21 to W-224; L-22 to W-224; W-23 to W-224; A-24 to W-224; W-25 to W-224; T-26 to W-224; L-27 to W-224; C-28 to W-224; G-29 to W-224; L-30 to W-224; C-31 to W-224; S-32 to W-224; L-33 to W-224; G-34 to W-224; A-35 to W-224; V-36 to W-224; G-37 to W-224; A-38 to W-224; P-39 to W-224; R-40 to W-224; P-41 to W-224; C-42 to W-224; Q-43 to W-224; A-44 to W-224; P-45 to W-224; Q-46 to W-224; Q-47 to W-224; W-48 to W-224; E-49 to W-224; G-50 to W-224; R-51 to W-224; Q-52 to W-224; V-53 to W-224; M-54 to W-224; Y-55 to W-224; Q-56 to W-224; Q-57 to W-224; S-58 to W-224; S-59 to W-224; G-60 to W-224; R-61 to W-224; N-62 to W-224; S-63 to W-224; R-64 to W-224; A-65 to W-224; L-66 to W-224; L-67 to W-224; S-68 to W-224; Y-69 to W-224; D-70 to W-224; G-71 to W-224; L-72 to W-224; N-73 to W-224; Q-74 to W-224; R-75 to W-224; V-76 to W-224; R-77 to W-224; V-78 to W-224; L-79 to W-224; D-80 to W-224; E-81 to W-224; R-82 to W-224; K-83 to W-224; A-84 to W-224; L-85 to W-224; I-86 to W-224; P-87 to W-224; C-88 to W-224; K-89 to W-224; R-90 to W-224; L-91 to W-224; F-92 to W-224; E-93 to W-224; Y-94 to W-224; I-95 to W-224; L-96 to W-224; L-97 to W-224; Y-98 to W-224; K-99 to W-224; D-100 to W-224;

G-101 to W-224; V-102 to W-224; M-103 to W-224; F-104 to W-224; Q-105 to W-224; I-106
to W-224; D-107 to W-224; Q-108 to W-224; A-109 to W-224; T-110 to W-224; K-111 to
W-224; Q-112 to W-224; C-113 to W-224; S-114 to W-224; K-115 to W-224; M-116 to W-224;
T-117 to W-224; L-118 to W-224; T-119 to W-224; Q-120 to W-224; P-121 to W-224; W-122
to W-224; D-123 to W-224; P-124 to W-224; L-125 to W-224; D-126 to W-224; I-127 to
W-224; P-128 to W-224; Q-129 to W-224; N-130 to W-224; S-131 to W-224; T-132 to W-224;
F-133 to W-224; E-134 to W-224; D-135 to W-224; Q-136 to W-224; Y-137 to W-224; S-138
to W-224; I-139 to W-224; G-140 to W-224; G-141 to W-224; P-142 to W-224; Q-143 to
W-224; E-144 to W-224; Q-145 to W-224; I-146 to W-224; T-147 to W-224; V-148 to W-224;
Q-149 to W-224; E-150 to W-224; W-151 to W-224; S-152 to W-224; D-153 to W-224; R-154
to W-224; K-155 to W-224; S-156 to W-224; A-157 to W-224; R-158 to W-224; S-159 to
W-224; Y-160 to W-224; E-161 to W-224; T-162 to W-224; W-163 to W-224; I-164 to W-224;
G-165 to W-224; I-166 to W-224; Y-167 to W-224; T-168 to W-224; V-169 to W-224; K-170
to W-224; D-171 to W-224; C-172 to W-224; Y-173 to W-224; P-174 to W-224; V-175 to
W-224; Q-176 to W-224; E-177 to W-224; T-178 to W-224; F-179 to W-224; T-180 to W-224;
I-181 to W-224; N-182 to W-224; Y-183 to W-224; S-184 to W-224; V-185 to W-224; I-186 to
W-224; L-187 to W-224; S-188 to W-224; T-189 to W-224; R-190 to W-224; F-191 to W-224;
F-192 to W-224; D-193 to W-224; I-194 to W-224; Q-195 to W-224; L-196 to W-224; G-197 to
W-224; I-198 to W-224; K-199 to W-224; D-200 to W-224; P-201 to W-224; S-202 to W-224;
V-203 to W-224; F-204 to W-224; T-205 to W-224; P-206 to W-224; P-207 to W-224; S-208 to
W-224; T-209 to W-224; C-210 to W-224; Q-211 to W-224; M-212 to W-224; A-213 to W-224;
Q-214 to W-224; L-215 to W-224; E-216 to W-224; K-217 to W-224; M-218 to W-224; and
S-219 to W-224 of the Human Ependymin amino acid sequence shown in Figures 1A, 1B, and
1C (which is identical to the sequence shown as SEQ ID NO:2, with the exception that the amino
acid residues in Figures 1A, 1B, and 1C are numbered consecutively from 1 through 224 from
the N-terminus to the C-terminus, while the amino acid residues in SEQ ID NO:2 are numbered
consecutively from -37 through 187 to reflect the position of the predicted signal peptide).
Polynucleotides encoding these polypeptides are also encompassed by the invention.

        Also as mentioned above, even if deletion of one or more amino acids from the
C-terminus of a protein results in modification of loss of one or more biological functions of the
protein, other biological activities may still be retained. Thus, the ability of the shortened
Human Ependymin mutein to induce and/or bind to antibodies which recognize the complete or
mature of the protein generally will be retained when less than the majority of the residues of the
complete or mature protein are removed from the C-terminus. Whether a particular polypeptide
lacking C-terminal residues of a complete protein retains such immunologic activities can
readily be determined by routine methods described herein and otherwise known in the art. It is
not unlikely that a Human Ependymin mutein with a large number of deleted C-terminal amino
acid residues may retain some biological or immungenic activities. In fact, peptides composed
of as few as six Human Ependymin amino acid residues may often evoke an immune response.

        Accordingly, the present invention further provides polypeptides having one or more
residues deleted from the carboxy terminus of the amino acid sequence of the Human

Ependymin shown in SEQ ID NO:2, up to the proline residue at position number 6, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues 1-m^2 of SEQ ID NO:2, where m^2 is an integer in the range of 6 to 224, and 6 is the position of the first residue from the C-terminus of the complete Human Ependymin polypeptide believed to be required for at least immunogenic activity of the Human Ependymin protein.

More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues M-1 to S-223; M-1 to C-222; M-1 to D-221; M-1 to E-220; M-1 to S-219; M-1 to M-218; M-1 to K-217; M-1 to E-216; M-1 to L-215; M-1 to Q-214; M-1 to A-213; M-1 to M-212; M-1 to Q-211; M-1 to C-210; M-1 to T-209; M-1 to S-208; M-1 to P-207; M-1 to P-206; M-1 to T-205; M-1 to F-204; M-1 to V-203; M-1 to S-202; M-1 to P-201; M-1 to D-200; M-1 to K-199; M-1 to I-198; M-1 to G-197; M-1 to L-196; M-1 to Q-195; M-1 to I-194; M-1 to D-193; M-1 to F-192; M-1 to F-191; M-1 to R-190; M-1 to T-189; M-1 to S-188; M-1 to L-187; M-1 to I-186; M-1 to V-185; M-1 to S-184; M-1 to Y-183; M-1 to N-182; M-1 to I-181; M-1 to T-180; M-1 to F-179; M-1 to T-178; M-1 to E-177; M-1 to Q-176; M-1 to V-175; M-1 to P-174; M-1 to Y-173; M-1 to C-172; M-1 to D-171; M-1 to K-170; M-1 to V-169; M-1 to T-168; M-1 to Y-167; M-1 to I-166; M-1 to G-165; M-1 to I-164; M-1 to W-163; M-1 to T-162; M-1 to E-161; M-1 to Y-160; M-1 to S-159; M-1 to R-158; M-1 to A-157; M-1 to S-156; M-1 to K-155; M-1 to R-154; M-1 to D-153; M-1 to S-152; M-1 to W-151; M-1 to E-150; M-1 to Q-149; M-1 to V-148; M-1 to T-147; M-1 to I-146; M-1 to Q-145; M-1 to E-144; M-1 to Q-143; M-1 to P-142; M-1 to G-141; M-1 to G-140; M-1 to I-139; M-1 to S-138; M-1 to Y-137; M-1 to Q-136; M-1 to D-135; M-1 to E-134; M-1 to F-133; M-1 to T-132; M-1 to S-131; M-1 to N-130; M-1 to Q-129; M-1 to P-128; M-1 to I-127; M-1 to D-126; M-1 to L-125; M-1 to P-124; M-1 to D-123; M-1 to W-122; M-1 to P-121; M-1 to Q-120; M-1 to T-119; M-1 to L-118; M-1 to T-117; M-1 to M-116; M-1 to K-115; M-1 to S-114; M-1 to C-113; M-1 to Q-112; M-1 to K-111; M-1 to T-110; M-1 to A-109; M-1 to Q-108; M-1 to D-107; M-1 to I-106; M-1 to Q-105; M-1 to F-104; M-1 to M-103; M-1 to V-102; M-1 to G-101; M-1 to D-100; M-1 to K-99; M-1 to Y-98; M-1 to L-97; M-1 to L-96; M-1 to I-95; M-1 to Y-94; M-1 to E-93; M-1 to F-92; M-1 to L-91; M-1 to R-90; M-1 to K-89; M-1 to C-88; M-1 to P-87; M-1 to I-86; M-1 to L-85; M-1 to A-84; M-1 to K-83; M-1 to R-82; M-1 to E-81; M-1 to D-80; M-1 to L-79; M-1 to V-78; M-1 to R-77; M-1 to V-76; M-1 to R-75; M-1 to Q-74; M-1 to N-73; M-1 to L-72; M-1 to G-71; M-1 to D-70; M-1 to Y-69; M-1 to S-68; M-1 to L-67; M-1 to L-66; M-1 to A-65; M-1 to R-64; M-1 to S-63; M-1 to N-62; M-1 to R-61; M-1 to G-60; M-1 to S-59; M-1 to S-58; M-1 to Q-57; M-1 to Q-56; M-1 to Y-55; M-1 to M-54; M-1 to V-53; M-1 to Q-52; M-1 to R-51; M-1 to G-50; M-1 to E-49; M-1 to W-48; M-1 to Q-47; M-1 to Q-46; M-1 to P-45; M-1 to A-44; M-1 to Q-43; M-1 to C-42; M-1 to P-41; M-1 to R-40; M-1 to P-39; M-1 to A-38; M-1 to G-37; M-1 to V-36; M-1 to A-35; M-1 to G-34; M-1 to L-33; M-1 to S-32; M-1 to C-31; M-1 to L-30; M-1 to G-29; M-1 to C-28; M-1 to L-27; M-1 to T-26; M-1 to W-25; M-1 to A-24; M-1 to W-23; M-1 to L-22; M-1 to G-21; M-1 to G-20; M-1 to L-19; M-1 to L-18; M-1 to W-17; M-1 to A-16; M-1 to G-15;

M-1 to L-14; M-1 to A-13; M-1 to G-12; M-1 to P-11; M-1 to V-10; M-1 to T-9; M-1 to R-8;
M-1 to L-7; M-1 to P-6 of the sequence of the Human Ependymin sequence shown in Figures
1A, 1B, and 1C (which is identical to the sequence shown as SEQ ID NO:2, with the exception
that the amino acid residues in Figures 1A, 1B, and 1C are numbered consecutively from 1

5   through 224 from the N-terminus to the C-terminus, while the amino acid residues in SEQ ID
NO:2 are numbered consecutively from -37 through 187 to reflect the position of the predicted
signal peptide). Polynucleotides encoding these polypeptides also are provided.

The invention also provides polypeptides having one or more amino acids deleted from
both the amino and the carboxyl termini of a Human Ependymin polypeptide, which may be

10   described generally as having residues n^2-m^2 of Figures 1A, 1B, and 1C (SEQ ID NO:2), where
n^2 and m^2 are integers as described above.

Other Mutants

In addition to terminal deletion forms of the protein discussed above, it also will be
recognized by one of ordinary skill in the art that some amino acid sequences of the Ependymin

15   polypeptide can be varied without significant effect of the structure or function of the protein.
If such differences in sequence are contemplated, it should be remembered that there will be
critical areas on the protein which determine activity.

Thus, the invention further includes variations of the Ependymin polypeptide which
show substantial Ependymin polypeptide activity or which include regions of Ependymin

20   protein such as the protein portions discussed below. Such mutants include deletions, insertions,
inversions, repeats, and type substitutions selected according to general rules known in the art so
as have little effect on activity. For example, guidance concerning how to make phenotypically
silent amino acid substitutions is provided wherein the authors indicate that there are two main
approaches for studying the tolerance of an amino acid sequence to change (Bowie, J. U., *et al.*,

25   *Science* **247**:1306-1310 (1990)),. The first method relies on the process of evolution, in which
mutations are either accepted or rejected by natural selection. The second approach uses genetic
engineering to introduce amino acid changes at specific positions of a cloned gene and
selections or screens to identify sequences that maintain functionality.

As the authors state, these studies have revealed that proteins are surprisingly tolerant of

30   amino acid substitutions. The authors further indicate which amino acid changes are likely to be
permissive at a certain position of the protein. For example, most buried amino acid residues
require nonpolar side chains, whereas few features of surface side chains are generally
conserved. Other such phenotypically silent substitutions are described by Bowie and coworkers
(*supra*) and the references cited therein. Typically seen as conservative substitutions are the

35   replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile;
interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu,
substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and
Arg and replacements among the aromatic residues Phe, Tyr.

Thus, the fragment, derivative or analog of the polypeptide of SEQ ID NO:2, or that

40   encoded by the deposited cDNA, may be (i) one in which one or more of the amino acid

residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the above form of the polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the above form of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Thus, the Ependymin of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation. As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table II).

TABLE II. Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

Amino acids in the Ependymin protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or in vitro proliferative activity.

Of special interest are substitutions of charged amino acids with other charged or neutral amino acids which may produce proteins with highly desirable improved characteristics, such as less aggregation. Aggregation may not only reduce activity but also be problematic when preparing pharmaceutical formulations, because aggregates can be immunogenic (Pinckard, et

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al., *Clin. Exp. Immunol.* 2:331-340 (1967); Robbins, *et al., Diabetes* 36:838-845 (1987);

Cleland, *et al., Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993)).

Replacement of amino acids can also change the selectivity of the binding of a ligand to

cell surface receptors (for example, Ostade, *et al., Nature* 361:266-268 (1993)) describes certain

5   mutations resulting in selective binding of TNF-α to only one of the two known types of TNF

receptors. Sites that are critical for ligand-receptor binding can also be determined by structural

analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith, *et*

al., J. Mol. Biol. 224:899-904 (1992); de Vos, *et al. Science* 255:306-312 (1992)).

As described above, ependymin function relies, in part, on asparigine-linked

10   glycosylation. Although the relative locations of the two potential N-linked glycosylation sites

of Ependymin of the present invention are not conserved with respect to the other known

ependymins, it is likely that disruption of the either or both of the Ependymin glycosylation

sites will, at least, modulate Ependymin activity. As a result, mutation of the asparagine amino

acid residues at positions 93 and 145 of SEQ ID NO:2 or the threonine or serine amino acid

15   residues at positions 95 and 147, respectively, will, at least modulate the biological activity of

Ependymin of the present invention.

In addition, as depicted in Figures 2 and 3, five cysteine amino acid residues are

conserved between Ependymin of the present invention and five piscine ependymin homologs.

Since cysteine amino acid residues often function in a structural capacity with respect to the

20   tertiary structure of a polypeptide (or the quaternary structure of the same polypeptide in cases

where polymerization is expected), it is expected that mutation of any or all of the four

conserved cysteine amino acid residues will result in, at least, modulation of the biological

activity of Ependymin of the present invention. As shown in Figures 3A and 3B, the conserved

cysteine residues are located at positions 5, 51, 76, 135, and 173 of SEQ ID NO:2.

25   Similarly, several additional amino acids are highly conserved between Ependymin of

the present invention and the five piscine ependymins presented in Figures 3A and 3B. These

are Proline-8, Tyrosine-32, Aspartic Acid-33, Glycine-64, Isoleucine-69, Aspartic Acid-70,

Lysine-78, Leucine-81, Proline-91, Glycine-103, Tryptophan-114, Threonine-131,

Phenylalanine-167, Proline-170, and Glutamic Acid-179. It is expected that mutation of one,

30   several, or all of these amino acid residues will modulate the biological activity of Ependymin of

the present invention.

Mutations which may also modulate, and are less likely to completely eliminate, the

biological activity of Ependymin of the present invention may also be made by changing one or

more non-conserved amino acid residues throughout the Ependymin polypeptide. Also

35   forming part of the present invention are isolated polynucleotides comprising nucleic acid

sequences which encode the each of the above Ependymin mutants.

The polypeptides of the present invention are preferably provided in an isolated form,

and preferably are substantially purified. A recombinantly produced version of the Ependymin

polypeptide can be substantially purified by the one-step method described by Smith and

40   Johnson (*Gene* 67:31-40 (1988)). Polypeptides of the invention also can be purified from

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natural or recombinant sources using anti-Ependymin antibodies of the invention in methods which are well known in the art of protein purification.

The invention further provides an isolated Ependymin polypeptide comprising an amino acid sequence selected from the group consisting of: (a) the amino acid sequence of the full-length Ependymin polypeptide having the complete amino acid sequence shown in SEQ ID NO:2 (i.e., positions -37 to 187 of SEQ ID NO:2); (b) the amino acid sequence of the full-length Ependymin polypeptide having the complete amino acid sequence shown in SEQ ID NO:2 excepting the N-terminal methionine (i.e., positions -36 to 187 of SEQ ID NO:2); (c) the amino acid sequence of the predicted mature Ependymin polypeptide having the amino acid sequence at positions 1 to 187 in SEQ ID NO:2; (d) the complete amino acid sequence encoded by the cDNA clone contained in the ATCC Deposit No. 209464; (e) the complete amino acid sequence excepting the N-terminal methionine encoded by the cDNA clone contained in the ATCC Deposit No. 209464; and (f) the complete amino acid sequence of the predicted mature Ependymin polypeptide encoded by the cDNA clone contained in the ATCC Deposit No. 209464. The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 80% identical, more preferably at least 90% identical, and still more preferably 95%, 96%, 97%, 98% or 99% identical to those described in (a), (b), (c), (d), (e) or (f), above, as well as polypeptides having an amino acid sequence with at least 90% similarity, and more preferably at least 95% similarity, to those above.

Further polypeptides of the present invention include polypeptides which have at least 90% similarity, more preferably at least 95% similarity, and still more preferably at least 96%, 97%, 98% or 99% similarity to those described above. The polypeptides of the invention also comprise those which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptide encoded by the deposited cDNA or to the polypeptide of SEQ ID NO:2, and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

A further embodiment of the invention relates to a peptide or polypeptide which comprises the amino acid sequence of a Ependymin polypeptide having an amino acid sequence which contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, even more preferably, not more than 40 conservative amino acid substitutions, still more preferably, not more than 30 conservative amino acid substitutions, and still even more preferably, not more than 20 conservative amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of a Ependymin polypeptide, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

By "% similarity" for two polypeptides is intended a similarity score produced by comparing the amino acid sequences of the two polypeptides using the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711) and the default settings for determining similarity. Bestfit uses the local homology algorithm of Smith and Waterman

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(Advances in Applied Mathematics 2:482-489, 1981) to find the best segment of similarity between two sequences.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a Ependymin polypeptide is intended that the amino acid
5   sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the Ependymin polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with
10   another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups
15   within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in Figures 1A, 1B, and 1C (SEQ ID NO:2), the amino acid sequence encoded by deposited cDNA clone HDPIE88, or fragments thereof, can be determined conventionally using known computer programs such the
20   Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full
25   length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al.
30   (Comp. App. Biosci. 6:237-245 (1990)). Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter
35   than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of
40   residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query

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sequence. A determination of whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of this
5 embodiment. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence. For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The
10 deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly
15 matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of
20 the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

The polypeptide of the present invention could be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those
25 of skill in the art.

As described in detail below, the polypeptides of the present invention can also be used to raise polyclonal and monoclonal antibodies, which are useful in assays for detecting Ependymin protein expression as described below or as agonists and antagonists capable of enhancing or inhibiting Ependymin protein function. Further, such polypeptides can be used in
30 the yeast two-hybrid system to "capture" Ependymin protein binding proteins which are also candidate agonists and antagonists according to the present invention. The yeast two hybrid system is described by Fields and Song (*Nature* **340**:245-246 (1989)).

Epitope-Bearing Portions

In another aspect, the invention provides a peptide or polypeptide comprising an
35 epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic

epitopes of a protein generally is less than the number of antigenic epitopes (see, for instance, Geysen, et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983)).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein (see, for instance, Sutcliffe, J. G., et al., Science 219:660-666 (1983)). Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals. Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention (see, for instance, Wilson, et al., Cell 37:767-778 (1984)).

Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. Non-limiting examples of antigenic polypeptides or peptides that can be used to generate Ependymin-specific antibodies include: a polypeptide comprising amino acid residues from about Ala-1 to about Gln-9 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Pro-8 to about Val-16 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Gln-19 to about Arg-27 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Ile-69 to about Ser-77 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Asp-86 to about Glu-107 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Glu-113 to about Tyr-123 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Thr-131 to about Gln-139 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Leu-159 to about Phe-167 in SEQ ID NO:2; and a polypeptide comprising amino acid residues from about Leu-178 to about Ser-186 in SEQ ID NO:2. These polypeptide fragments have been determined to bear antigenic epitopes of the Ependymin protein by the analysis of the Jameson-Wolf antigenic index, as shown in Figure 4 and Table I, above.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means (see, for example, Houghten, R. A., et al., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985); and U.S. Patent No. 4,631,211 to Houghten, et al. (1986)).

Epitope-bearing peptides and polypeptides of the invention are used to induce antibodies according to methods well known in the art (see, for instance, Sutcliffe, et al., supra; Wilson, et al., supra; Chow, M., et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle, F. J., et al., J. Gen. Virol. 66:2347-2354 (1985)). Immunogenic epitope-bearing peptides of the invention, i.e., those parts of a protein that elicit an antibody response when the whole protein is the immunogen, are identified according to methods known in the art (see, for instance, Geysen, et al., supra). Further still, U.S. Patent No. 5,194,392, issued to Geysen, describes a general method of detecting or determining the sequence of monomers (amino acids or other

compounds) which is a topological equivalent of the epitope (i.e., a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Patent No. 4,433,092, issued to Geysen, describes a method of detecting or determining a sequence of monomers which is a topographical equivalent of a ligand which is
5   complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Patent No. 5,480,971, issued to Houghten and colleagues, on Peralkylated Oligopeptide Mixtures discloses linear C1-C7-alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of
10   interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods.

Fusion Proteins

As one of skill in the art will appreciate, Ependymin polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with
15   parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EP A 394,827; Traunecker, *et al., Nature* **331**:84-86 (1988)).
20   Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric Ependymin protein or protein fragment alone (Fountoulakis, *et al., J. Biochem.* **270**:3958-3964 (1995)).

Antibodies

Ependymin protein-specific antibodies for use in the present invention can be raised
25   against the intact Ependymin protein or an antigenic polypeptide fragment thereof, which may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')2
30   fragments) which are capable of specifically binding to Ependymin protein. Fab and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl, *et al., J. Nucl. Med.* **24**:316-325 (1983)). Thus, these fragments are preferred.

The antibodies of the present invention may be prepared by any of a variety of methods.
35   For example, cells expressing the Ependymin protein or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of Ependymin protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

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In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or Ependymin protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology (Kohler, *et al., Nature* **256**:495 (1975); Kohler, *et al., Eur. J. Immunol.* **6**:511 (1976); Kohler, *et al., Eur. J. Immunol.* **6**:292 (1976); Hammerling, *et al., in: Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., (1981) pp. 563-681)).
In general, such procedures involve immunizing an animal (preferably a mouse) with a Ependymin protein antigen or, more preferably, with a Ependymin protein-expressing cell. Suitable cells can be recognized by their capacity to bind anti-Ependymin protein antibody. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56° C), and supplemented with about 10 μg/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 μg/ml of streptomycin. The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the American Type Culture Collection, Manassas, Virginia. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands and colleagues (*Gastroenterology* **80**:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the Ependymin protein antigen.

Alternatively, additional antibodies capable of binding to the Ependymin protein antigen may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and that, therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, Ependymin protein-specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the Ependymin protein-specific antibody can be blocked by the Ependymin protein antigen. Such antibodies comprise anti-idiotypic antibodies to the Ependymin protein-specific antibody and can be used to immunize an animal to induce formation of further Ependymin protein-specific antibodies.

It will be appreciated that Fab and F(ab')2 and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). Alternatively, Ependymin protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For *in vivo* use of anti-Ependymin in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art (Morrison, *Science* **229**:1202 (1985);

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Oi, *et al.*, *BioTechniques* 4:214 (1986); Cabilly, *et al.*, U.S. Patent No. 4,816,567; Taniguchi, *et al.*, EP 171496; Morrison, *et al.*, EP 173494; Neuberger, *et al.*, WO 8601533; Robinson, *et al.*, WO 8702671; Boulianne, *et al.*, *Nature* 312:643 (1984); Neuberger, *et al.*, *Nature* 314:268 (1985).

Nervous System-Related Disorders

Diagnosis

The present inventors have discovered that Ependymin is expressed in primary dendritic cells, the KMH2 cell line, placenta, fetal and adult liver, spinal cord, osteoclastoma, cerebellum, synovial fibroblasts, 12 week old early stage human embryo, adrenal gland tumor, whole brain, Hodgkin's Lymphoma tissue, macrophages, HEL cell line, and chondrosarcoma. For a number of nervous system-related disorders, substantially altered (increased or decreased) levels of Ependymin gene expression can be detected in nervous system tissue or other cells or bodily fluids (e.g., sera, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" Ependymin gene expression level, that is, the Ependymin expression level in nervous system tissues or bodily fluids from an individual not having the nervous system disorder. Thus, the invention provides a diagnostic method useful during diagnosis of a nervous system disorder, which involves measuring the expression level of the gene encoding the Ependymin protein in nervous system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard Ependymin gene expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of an nervous system disorder.

In particular, it is believed that certain tissues in mammals with cancer of the nervous express significantly reduced levels of the Ependymin protein and mRNA encoding the Ependymin protein when compared to a corresponding "standard" level. Further, it is believed that enhanced levels of the Ependymin protein can be detected in certain body fluids (e.g., sera, plasma, urine, and spinal fluid) from mammals with such a cancer when compared to sera from mammals of the same species not having the cancer.

Thus, the invention provides a diagnostic method useful during diagnosis of a nervous system disorder, including cancers of this system, which involves measuring the expression level of the gene encoding the Ependymin protein in nervous system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard Ependymin gene expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a nervous system disorder.

Where a diagnosis of a disorder in the nervous system, including diagnosis of a tumor, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting depressed Ependymin gene expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "assaying the expression level of the gene encoding the Ependymin protein" is intended qualitatively or quantitatively measuring or estimating the level of the Ependymin

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protein or the level of the mRNA encoding the Ependymin protein in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the Ependymin protein level or mRNA level in a second biological sample). Preferably, the Ependymin protein level or mRNA level in the first

5   biological sample is measured or estimated and compared to a standard Ependymin protein level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder of the nervous system. As will be appreciated in the art, once a standard Ependymin protein level or mRNA level is known, it can be used repeatedly as a

10   standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains Ependymin protein or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain free Ependymin protein, nervous system tissue, and other tissue

15   sources found to express complete or mature Ependymin or a Ependymin receptor. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The present invention is useful for diagnosis or treatment of various nervous system-related disorders in mammals, preferably humans. Such disorders include any disregulations of

20   nervous cell function including, but not limited to, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, pain, stroke, depression, anxiety, epilepsy, other neurological and psychiatric disorders, and the like.

Total cellular RNA can be isolated from a biological sample using any suitable technique such as the single-step guanidinium-thiocyanate-phenol-chloroform method

25   described by Chomczynski and Sacchi (*Anal. Biochem.* 162:156-159 (1987)). Levels of mRNA encoding the Ependymin protein are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

30   Assaying Ependymin protein levels in a biological sample can occur using antibody-based techniques. For example, Ependymin protein expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al., J. Cell. Biol.* 101:976-985 (1985); Jalkanen, M., *et al., J. Cell. Biol.* 105:3087-3096 (1987)). Other antibody-based methods useful for detecting Ependymin protein gene expression include

35   immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

40   In addition to assaying Ependymin protein levels in a biological sample obtained from an individual, Ependymin protein can also be detected *in vivo* by imaging. Antibody labels or

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markers for *in vivo* imaging of Ependymin protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium,

5   which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

     A Ependymin protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{112}In, ^{99m}Tc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is

10   introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc.

15   The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain Ependymin protein. *In vivo* tumor imaging is described by Burchiel and coworkers (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, Burchiel, S. W. and Rhodes, B. A., eds., Masson Publishing Inc. (1982)).

20   ***Treatment***

     As noted above, Ependymin polynucleotides and polypeptides are useful for diagnosis of conditions involving abnormally high or low expression of Ependymin activities. Given the cells and tissues where Ependymin is expressed as well as the activities modulated by Ependymin, it is readily apparent that a substantially altered (increased or decreased) level of

25   expression of Ependymin in an individual compared to the standard or "normal" level produces pathological conditions related to the bodily system(s) in which Ependymin is expressed and/or is active.

     It is well-known in the art that, in addition to a specific cellular function, cellular receptor molecules may also often be exploited by a virus as a means of initiating entry into a potential

30   host cell. For example, it was recently discovered by Wu and colleagues (*J. Exp. Med.* **185**:1681-1691 (1997)) that the cellular chemokine receptor CCR5 functions not only as a cellular chemokine receptor, but also as a receptor for macrophage-tropic human immunodeficiency virus (HIV)-1. In addition, RANTES, MIP-1a, and MIP-1b, which are agonists for the cellular chemokine receptor CCR5, inhibit entry of various strains of HIV-1 into

35   susceptible cell lines (Cocchi, F., *et al.*, *Science* **270**:1811-1815 (1995)). Thus, the invention also provides a method of treating an individual exposed to, or infected with, a virus through the prophylactic or therapeutic administration of Ependymin, or an agonist or antagonist thereof, to block or disrupt the interaction of a viral particle with the Ependymin receptor and, as a result, block the initiation or continuation of viral infectivity.

40   The Ependymin of the present invention binds to the Ependymin receptor and, as such, is likely to block neurotropic viral infections. Further, Ependymin expression is also observed

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in many bone and cartilage tissues, and, as such, Ependymin is also likely to block initiation of infectious cycle of many viruses which infect bone or cartilage. More specifically, a non-limiting list of viruses whose infectious life cycles may be altered by Ependymin includes retroviruses such as HIV-1, HIV-2, human T-cell lymphotropic virus (HTLV)-I, and HTLV-II, as

5   well as other DNA and RNA viruses including herpes simplex virus (HSV)-1, HSV-2, HSV-6, cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes samirii, adenoviruses, rhinoviruses, influenza viruses, reoviruses, and the like.

The ability of Ependymin of the present invention, or agonists or antagonists thereof, to prophylactically or therapeutically block viral infection may be easily tested by the skilled

10   artisan. For example, Simmons and coworkers (Science 276:276-279 (1997)) and Arenzana-Seisdedos and colleagues (*Nature* 383:400 (1996)) each outline a method of observing suppression of HIV-1 infection by an antagonist of the CCR5 chemokine receptor and of the CC chemokine RANTES, respectively, in cultured peripheral blood mononuclear cells. Cells are cultured and infected with a virus, HIV-1 in both cases noted above. An agonist

15   or antagonist of the CC chemokine or its receptor is then immediately added to the culture medium. Evidence of the ability of the agonist or antagonist of the chemokine or cellular receptor is determined by evaluating the relative success of viral infection at 3, 6, and 9 days postinfection.

Administration of a pharmaceutical composition comprising an amount of an isolated

20   Ependymin, or an agonist or antagonist thereof, of the invention to an individual either infected with a virus or at risk for infection with a virus is performed as described below.

It will also be appreciated by one of ordinary skill that, since the Ependymin protein of the invention is a member of the ependymin polypeptide family, the mature secreted form of the protein may be released in soluble form from the cells which express the Ependymin by

25   proteolytic cleavage. Therefore, when Ependymin mature form is added from an exogenous source to cells, tissues or the body of an individual, the protein will exert its physiological activities on its target cells of that individual.

Therefore, it will be appreciated that conditions caused by a decrease in the standard or normal level of Ependymin activity in an individual, particularly disorders of the nervous

30   system, can be treated by administration of Ependymin polypeptide (in the form of the mature protein). Thus, the invention also provides a method of treatment of an individual in need of an increased level of Ependymin activity comprising administering to such an individual a pharmaceutical composition comprising an amount of an isolated Ependymin polypeptide of the invention, particularly a mature form of the Ependymin protein of the invention, effective to

35   increase the Ependymin activity level in such an individual.

Ependymin is found bound to collagen fibrils in the extracellular space of the mammalian leptomeninges. Ependymin, or agonists or antagonists thereof, may thus be employed in the treatment of ependymitis or other inflammation of the cellular membrane lining the central canal of the spinal cord and the brain ventricles. Ependymin is also found in

40   collagen fibrils covering the endothelial cells of numerous blood vessels. Similarly, Ependymin, or agonists or antagonists thereof, may be used to regulate angiogenesis, and other processes or

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disorders which involve the formation, maintenance or disorganization of blood vessels, and to treat a variety of cancers which involve the formation of new blood vessels. A list of such cancers may include, but is not limited by, breast cancer, colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer,

5   testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, adenoma, and the like.

Ependymins and other antiadhesive extracellular matrix proteins are involved in the mechanism whereby meningeal cells influence endfoot formation of Bergmann glial cells, which organize the superficial glia limitans surrounding the CNS. This function is essential in

10   establishing accurate early development of the CNS and, as such, Ependymin of the present invention, or agonists or antagonists thereof, may be used to treat developmental disorders of the CNS and the brain. Ependymin may also be used to treat additional neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration, and the like. Further, Ependymin of the present

15   invention, or agonists or antagonists thereof, may be used to modulate long-term memory consolidation.

Ependymin of the present invention, or agonists or antagonists thereof, may be used to enhance the regeneration of the optic or other nerves. Several scientific studies have revealed that goldfish ependymin expression increases during optic nerve regeneration and that

20   anti-ependymin antibodies can prevent the sharpening of the regenerating retinotectal projection (Schmidt, R. and Shashoua, V. E. *J. Neurochem.* **36**:1368-1377 (1988); Thomodsson, F. R., *et al.*, *Exp. Neurol.* **117**:260-268 (1992)).

Ependymin of the present invention, or agonists or antagonists thereof, may be used to treat disorders resulting from axon ingrowth, for example, along blood vessels or into the

25   meninx, or prophylactically, to prevent undesired or incorrect neuronal growth.

Ependymin of the present invention, or agonists or antagonists thereof, may be used to treat disorders of the blood-brain barrier since ependymin participates in the endothelial cell barrier by modulating cell-matrix interactions.

30   *Formulations*

The Ependymin polypeptide composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with Ependymin polypeptide alone), the site of delivery of the Ependymin polypeptide composition, the method of administration, the

35   scheduling of administration, and other factors known to practitioners. The "effective amount" of Ependymin polypeptide for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of Ependymin polypeptide administered parenterally per dose will be in the range of about 1 μg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic

40   discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the

Ependymin polypeptide is typically administered at a dose rate of about 1 µg/kg/hour to about 50 µg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing the Ependymin of the invention may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The Ependymin polypeptide is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U., et al., Biopolymers 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate; Langer, R., et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, R., Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer, R., et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release Ependymin polypeptide compositions also include liposomally entrapped Ependymin polypeptide. Liposomes containing Ependymin polypeptide are prepared by methods known in the art (DE 3,218,121; Epstein, et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang, et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324). Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Ependymin polypeptide therapy.

For parenteral administration, in one embodiment, the Ependymin polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting the Ependymin polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution.

Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Ependymin polypeptide is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of Ependymin polypeptide salts.

Ependymin polypeptide to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic Ependymin polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Ependymin polypeptide ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Ependymin polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Ependymin polypeptide using bacteriostatic water-for-injection (WFI).

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

Agonists and Antagonists - Assays and Molecules

The invention also provides a method of screening compounds to identify those which enhance or block the action of Ependymin on cells, such as its interaction with Ependymin-binding molecules such as receptor molecules. An agonist is a compound which increases the

natural biological functions of Ependymin or which functions in a manner similar to
Ependymin, while antagonists decrease or eliminate such functions.

In another aspect of this embodiment the invention provides a method for identifying a
receptor protein or other ligand-binding protein which binds specifically to a Ependymin

5   polypeptide. For example, a cellular compartment, such as a membrane or a preparation
thereof, may be prepared from a cell that expresses a molecule that binds Ependymin. The
preparation is incubated with labeled Ependymin and complexes of Ependymin bound to the
receptor or other binding protein are isolated and characterized according to routine methods
known in the art. Alternatively, the Ependymin polypeptide may be bound to a solid support so

10   that binding molecules solubilized from cells are bound to the column and then eluted and
characterized according to routine methods.

In the assay of the invention for agonists or antagonists, a cellular compartment, such as
a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that
binds Ependymin, such as a molecule of a signaling or regulatory pathway modulated by

15   Ependymin. The preparation is incubated with labeled Ependymin in the absence or the
presence of a candidate molecule which may be a Ependymin agonist or antagonist. The ability
of the candidate molecule to bind the binding molecule is reflected in decreased binding of the
labeled ligand. Molecules which bind gratuitously, i.e., without inducing the effects of
Ependymin on binding the Ependymin binding molecule, are most likely to be good

20   antagonists. Molecules that bind well and elicit effects that are the same as or closely related to
Ependymin are agonists.

Ependymin-like effects of potential agonists and antagonists may by measured, for
instance, by determining activity of a second messenger system following interaction of the
candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that

25   of Ependymin or molecules that elicit the same effects as Ependymin. Second messenger
systems that may be useful in this regard include but are not limited to AMP guanylate cyclase,
ion channel or phosphoinositide hydrolysis second messenger systems.

Another example of an assay for Ependymin antagonists is a competitive assay that
combines Ependymin and a potential antagonist with membrane-bound Ependymin receptor

30   molecules or recombinant Ependymin receptor molecules under appropriate conditions for a
competitive inhibition assay. Ependymin can be labeled, such as by radioactivity, such that the
number of Ependymin molecules bound to a receptor molecule can be determined accurately to
assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and

35   antibodies that bind to a polypeptide of the invention and thereby inhibit or extinguish its
activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide
such as a closely related protein or antibody that binds the same sites on a binding molecule,
such as a receptor molecule, without inducing Ependymin-induced activities, thereby preventing
the action of Ependymin by excluding Ependymin from binding.

40   Other potential antagonists include antisense molecules. Antisense technology can be
used to control gene expression through antisense DNA or RNA or through triple-helix

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formation. Antisense techniques are discussed in a number of studies (for example, Okano, *J. Neurochem.* **56**:560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression." CRC Press, Boca Raton, FL (1988)). Triple helix formation is discussed in a number of studies, as well (for instance, Lee, *et al., Nucleic Acids Research* **6**:3073 (1979);

5   Cooney, *et al., Science* **241**:456 (1988); Dervan, *et al., Science* **251**:1360 (1991)). The methods are based on binding of a polynucleotide to a complementary DNA or RNA. For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of

10   the gene involved in transcription thereby preventing transcription and the production of Ependymin. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into Ependymin polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of Ependymin protein.

15   The agonists and antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described above.

               The antagonists may be employed for instance to inhibit the formation of Ependymin-collagen fibrils which typically cover the endothelial cells of numerous blood vessels. As a result, anti-Ependymin antibodies may be used to regulate angiogenesis, and other

20   processes or disorders which involve the formation, maintenance or disorganization of blood vessels, and to treat a variety of cancers which involve the formation of new blood vessels. Antibodies against Ependymin may also be employed to bind to and inhibit Ependymin activity to treat Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, pain, stroke, depression, anxiety, epilepsy, and other neurological and psychiatric disorders. Any of the

25   above antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

Gene Mapping

               The nucleic acid molecules of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular

30   location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

35   In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of a Ependymin protein gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for *in situ* chromosome mapping using well known techniques for this purpose.

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        In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR
primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region
of the gene is used to rapidly select primers that do not span more than one exon in the genomic
DNA, thus complicating the amplification process. These primers are then used for PCR
5   screening of somatic cell hybrids containing individual human chromosomes. Fluorescence *in
situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to
provide a precise chromosomal location in one step. This technique can be used with probes
from the cDNA as short as 50 or 60 bp (for a review of this technique, see Verma, *et al., Human
Chromosomes: A Manual Of Basic Techniques*, Pergamon Press, New York (1988)).

10   Once a sequence has been mapped to a precise chromosomal location, the physical
position of the sequence on the chromosome can be correlated with genetic map data. Such
data are found, for example, on the World Wide Web (McKusick, V. *Mendelian Inheritance In
Man*, available on-line through Johns Hopkins University, Welch Medical Library). The
relationship between genes and diseases that have been mapped to the same chromosomal region
15   are then identified through linkage analysis (coinheritance of physically adjacent genes).

        Next, it is necessary to determine the differences in the cDNA or genomic sequence
between affected and unaffected individuals. If a mutation is observed in some or all of the
affected individuals but not in any normal individuals, then the mutation is likely to be the
causative agent of the disease.

20   Having generally described the invention, the same will be more readily understood by
reference to the following examples, which are provided by way of illustration and are not
intended as limiting.

Examples
Example 1(a): Expression and Purification of "His-tagged" Ependymin in E. coli

25   The novel pHE4 series of bacterial expression vectors, in particular, the pHE4-5 vector
may be used for bacterial expression in this example. pHE4-5/MPIFD23 vector plasmid DNA
contains an insert which encodes another ORF. The construct was deposited with the American
Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, on
September 30, 1997 and given Accession No. 209311. Using the *Nde* I and *Asp* 718 restriction
30   sites flanking the irrelevant MPIF ORF insert, the pHE4-5 is linearized and the MPIF ORF is
removed.

        The pHE4-5 bacterial expression vector includes a neomycin phosphotransferase gene
for selection, an *E. coli* origin of replication, a T5 phage promoter sequence, two *lac* operator
sequences, a Shine-Delgarno sequence, and the lactose operon repressor gene (*lac*Iq). These
35   elements are arranged such that an inserted DNA fragment encoding a polypeptide expresses
that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the amino
terminus of that polypeptide. The promoter and operator sequences of the pHE4-5 vector were
made synthetically. Synthetic production of nucleic acid sequences is well known in the art

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(CLONETECH 95/96 Catalog, pages 215-216, CLONETECH, 1020 East Meadow Circle, Palo
Alto, CA 94303).

The DNA sequence encoding the desired portion of the Ependymin protein comprising
the mature form of the Ependymin amino acid sequence is amplified from the deposited cDNA
clone using PCR oligonucleotide primers which anneal to the amino terminal sequences of the
desired portion of the Ependymin protein and to sequences in the deposited construct 3' to the
cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning
in the pHE4-5 vector are added to the 5' and 3' primer sequences, respectively.

For cloning the mature form of the Ependymin protein, the 5' primer has the sequence
5' GCG CAT ATG GCC CCG CGC CCG TGC 3' (SEQ ID NO:8) containing the underlined *Nde*
I restriction site followed by 15 nucleotides of the amino terminal coding sequence of the
mature Ependymin sequence in SEQ ID NO:2. One of ordinary skill in the art would
appreciate, of course, that the point in the protein coding sequence where the 5' primer begins
may be varied to amplify a DNA segment encoding any desired portion of the complete
Ependymin protein shorter or longer than the mature form of the protein. The 3' primer has the
sequence 5' GCG GGT ACC TCA CCA GGA GCA GTC TTC GC 3' (SEQ ID NO:9) containing
the underlined *Asp* 718 restriction site followed by 20 nucleotides complementary to the 3' end
of the coding sequence of the Ependymin DNA sequence in Figures 1A, 1B, and 1C.

The amplified Ependymin DNA fragment and the vector pHE4-5 are digested with *Nde*
I and *Asp* 718 and the digested DNAs are then ligated together. Insertion of the Ependymin
DNA into the restricted pHE4-5 vector places the Ependymin protein coding region downstream
from the IPTG-inducible promoter and in-frame with an initiating AUG and the six histidine
codons.

The ligation mixture is transformed into competent *E. coli* cells using standard
procedures such as those described by Sambrook and colleagues (*Molecular Cloning: a
Laboratory Manual, 2nd Ed.*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
(1989)). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which
expresses the lac repressor and confers kanamycin resistance ("Kanr"), is used in carrying out
the illustrative example described herein. This strain, which is only one of many that are suitable
for expressing Ependymin protein, is available commercially (QIAGEN, Inc., *supra*).
Transformants are identified by their ability to grow on LB plates in the presence of ampicillin
and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned
DNA confirmed by restriction analysis, PCR and DNA sequencing.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in
LB media supplemented with both ampicillin (100 µg/ml) and kanamycin (25 µg/ml). The O/N
culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The
cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-β-
D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce

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transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

The cells are then stirred for 3-4 hours at 4°C in 6M guanidine-HCl, pH 8. The cell debris is removed by centrifugation, and the supernatant containing the Ependymin is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist, 1995, QIAGEN, Inc., *supra*). Briefly the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the Ependymin is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins can be eluted by the addition of 250 mM immidazole. Immidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4°C or frozen at -80°C.

The following alternative method may be used to purify Ependymin expressed in *E coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10°C and the cells are harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells ware then lysed by passing the solution through a microfluidizer (Microfuidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 x *g* for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 x *g* centrifugation for 15 min., the pellet is discarded and the Ependymin polypeptide-containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

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Following high speed centrifugation (30,000 x *g*) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

To clarify the refolded Ependymin polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 μm membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 mm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the Ependymin polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant A_{280} monitoring of the effluent. Fractions containing the Ependymin polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant Ependymin polypeptide exhibits greater than 95% purity after the above refolding and purification steps. No major contaminant bands are observed from Commassie blue stained 16% SDS-PAGE gel when 5 μg of purified protein is loaded. The purified protein is also tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

Example 2: Cloning and Expression of Ependymin protein in a Baculovirus Expression System

In this illustrative example, the plasmid shuttle vector pA2 is used to insert the cloned DNA encoding complete protein, including its naturally associated secretory signal (leader) sequence, into a baculovirus to express the mature Ependymin protein, using standard methods as described by Summers and colleagues (*A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987)). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as *Bam* HI, *Xba* I and *Asp* 718. The polyadenylation site of the simian virus 40 ("SV40") is used

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for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with
5   wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors could be used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described,
10   for instance, by Luckow and coworkers (*Virology* **170**:31-39 (1989)).

The cDNA sequence encoding the full length Ependymin protein in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence shown in SEQ ID NO:2, is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5' GAT CGC <u>TCT AGA</u> TCC GCC ACC
15   ATG CCA GGA CGC GCT CCC CTC CGC ACC GTC 3' (SEQ ID NO:10) containing the underlined *Xba* I restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells (Kozak, M., *J. Mol. Biol.* **196**:947-950 (1987)), followed by 27 of nucleotides of the sequence of the complete Ependymin protein shown in Figures 1A, 1B, and 1C, beginning with the AUG initiation codon. The 3' primer has the sequence 5' GAT CGC <u>GGT</u>
20   <u>ACC</u> TTA TCA CCA GGA GCA GTC TTC GCT CAT CTT CTC CAG 3' (SEQ ID NO:11) containing the underlined *Asp* 718 restriction site followed by 30 nucleotides complementary to the 3' noncoding sequence in Figures 1A, 1B, and 1C.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with *Xba*
25   I and *Asp* 718 and again is purified on a 1% agarose gel. This fragment is designated herein F1.

The plasmid is digested with the restriction enzymes *Xba* I and *Asp* 718 and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is designated herein "V1".

30   Fragment F1 and the dephosphorylated plasmid V1 are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Statagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria are identified that contain the plasmid with the human Ependymin gene by digesting DNA from individual colonies using *Xba* I and *Asp* 718 and then analyzing the
35   digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pA2Ependymin.

Five μg of the plasmid pA2Ependymin is co-transfected with 1.0 μg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San

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Diego, CA), using the lipofection method described by Felgner and colleagues (*Proc. Natl. Acad. Sci. USA* **84**:7413-7417 (1987)). One µg of BaculoGold™ virus DNA and 5 µg of the plasmid pA2Ependymin are mixed in a sterile well of a microtiter plate containing 50 µl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 µl

5   Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27°C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added.

10   Cultivation is then continued at 27°C for four days.

         After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith (*supra*). An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can

15   also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10). After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 µl of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells

20   seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. The recombinant virus is called V-Ependymin.

         To verify the expression of the Ependymin gene Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-Ependymin at a multiplicity of infection ("MOI") of about 2. If radiolabeled

25   proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 µCi of ^{35}S-methionine and 5 µCi ^{35}S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE

30   followed by autoradiography (if radiolabeled).

         Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the mature form of the Ependymin protein and thus the cleavage point and length of the naturally associated secretory signal peptide.

Example 3: Cloning and Expression of Ependymin in Mammalian Cells

A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS; Murphy, et al., Biochem J. 227:277-279 (1991); Bebbington, et al., Bio/Technology 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen, et al., Mol. Cel. Biol. 5:438-447 (1985)) plus a fragment of the CMV-enhancer (Boshart, et al., Cell 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites Bam HI, Xba I and Asp 718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Example 3(a): Cloning and Expression in COS Cells

The expression plasmid, pEpendyminHA, is made by cloning a portion of the cDNA encoding the complete Ependymin protein into the expression vector pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.).

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The expression vector pcDNAI/amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron; (5) several codons encoding a hemagglutinin fragment (i.e., an "HA" tag to facilitate purification) followed by a termination codon and polyadenylation signal arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson and colleagues (*Cell* 37:767 (1984)). The fusion of the HA tag to the target protein allows easy detection and recovery of the recombinant protein with an antibody that recognizes the HA epitope. pcDNAIII contains, in addition, the selectable neomycin marker.

A DNA fragment encoding the complete Ependymin polypeptide is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The plasmid construction strategy is as follows. The Ependymin cDNA of the deposited clone is amplified using primers that contain convenient restriction sites, much as described above for construction of vectors for expression of Ependymin in *E. coli*. Suitable primers include the following, which are used in this example. The 5' primer, containing the underlined *Asp* 718 site, a Kozak sequence, an AUG start codon, a sequence, and 24 nucleotides of the 5' coding region of the complete Ependymin polypeptide, has the following sequence: 5' GAT CGC GGT ACC GCC ATC ATG CCA GGA CGC GCT CCC CTC CGC 3' (SEQ ID NO:12). The 3' primer, containing the underlined *Bam* HI and 20 of nucleotides complementary to the 3' coding sequence immediately before the stop codon, has the following sequence: 5' GAT CGC GGA TCC TCA CCA GGA GCA GTC TTC GC 3' (SEQ ID NO:13).

The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with *Asp* 718 and *Bam* HI and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (Stratagene Cloning Systems, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis or other means for the presence of the fragment encoding the complete Ependymin polypeptide

For expression of recombinant Ependymin, COS cells are transfected with an expression vector, as described above, using DEAE-dextran, as described, for instance, by Sambrook and coworkers (*Molecular Cloning: a Laboratory Manual*, Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989)). Cells are incubated under conditions for expression of Ependymin by the vector.

Expression of the Ependymin-HA fusion protein is detected by radiolabeling and immunoprecipitation, using methods described in, for example Harlow and colleagues (*Antibodies: A Laboratory Manual, 2nd Ed.*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988)). To this end, two days after transfection, the cells are labeled by

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incubation in media containing ^{35}S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and the lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson and colleagues (supra). Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

Example 3(b): Cloning and Expression in CHO Cells

The vector pC4 is used for the expression of Ependymin polypeptide. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C. Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A. Biotechnology 9:64-68 (1991)). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach may be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rouse Sarcoma Virus (Cullen, et al., Mol. Cell. Biol. 5:438-447 (1985)) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV; Boshart, et al., Cell 41:521-530 (1985)). Downstream of the promoter are the following single restriction enzyme cleavage sites that allow the integration of the genes: Bam HI, Xba I, and Asp 718. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human ß-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the Ependymin polypeptide in a regulated way in mammalian cells (Gossen, M., and Bujard, H. Proc. Natl. Acad. Sci. USA 89:5547-5551 (1992)). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-

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transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with the restriction enzymes *Xba* I and *Asp* 718 and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the complete Ependymin polypeptide is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the desired portion of the gene. The 5' primer containing the underlined *Xba* I site, a Kozak sequence (in italics), an AUG start codon, and 30 nucleotides of the 5' coding region of the complete Ependymin polypeptide, has the following sequence: 5' GAT CGC TCT AGA *TCC GCC ACC* ATG CCA GGA CGC GCT CCC CTC CGC ACC GTC 3' (SEQ ID NO:14). The 3' primer, containing the underlined *Asp* 718 and 30 of nucleotides complementary to the 3' coding sequence immediately before the stop codon as shown in Figures 1A, 1B, and 1C (SEQ ID NO:1), has the sequence shown in the above example as SEQ ID NO:11.

The amplified fragment is digested with the endonucleases *Xba* I and *Asp* 718 and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene are used for transfection. Five µg of the expression plasmid pC4 is cotransfected with 0.5 µg of the plasmid pSVneo using lipofectin (Felgner, *et al., supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of metothrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 µM, 2 µM, 5 µM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100-200 µM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Example 4: Tissue distribution of Ependymin mRNA expression

Northern blot analysis is carried out to examine Ependymin gene expression in human tissues, using methods described by, among others, Sambrook and colleagues (*supra*). A cDNA

probe containing the entire nucleotide sequence of the Ependymin protein (SEQ ID NO:1) is labeled with ^{32}P using the *redi*prime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using a CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol

5   number PT1200-1. The purified labeled probe is then used to examine various human tissues for Ependymin mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's

10   protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and films developed according to standard procedures.

It will be clear that the invention may be practiced otherwise than as particularly

15   described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by

20   reference.

Further, the Sequence Listing submitted herewith, and the Sequence Listing submitted with U.S. Provisional Application Serial No. 60/071,330, filed on January 14, 1998, and the Sequence Listing submitted with U.S. Provisional Application Serial No. 60/075,278, filed on February 19, 1998 (to both of which the present application claims benefit of the filing date

25   under 35 U.S.C. § 119(e)), in both computer and paper forms are hereby incorporated by reference in their entireties.

What Is Claimed Is:

1.   An isolated nucleic acid molecule nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the Ependymin polypeptide having the complete amino acid sequence in SEQ ID NO:2 (i.e., positions -37 to 187 of SEQ ID NO:2);

(b) a nucleotide sequence encoding the Ependymin polypeptide having the complete amino acid sequence in SEQ ID NO:2 excepting the N-terminal methionine (i.e., positions -36 to 187 of SEQ ID NO:2);

(c) a nucleotide sequence encoding the predicted mature Ependymin polypeptide having the amino acid sequence at positions 1 to 187 in SEQ ID NO:2;

(d) a nucleotide sequence encoding the Ependymin polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209464;

(e) a nucleotide sequence encoding the Ependymin polypeptide having the complete amino acid sequence excepting the N-terminal methionine encoded by the cDNA clone contained in ATCC Deposit No. 209464;

(f) a nucleotide sequence encoding the mature Ependymin polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209464; and

(g) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e) or (f), above.

2.   The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence in Figures 1A, 1B, and 1C (SEQ ID NO:1).

3.   The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figures 1A, 1B, and 1C (SEQ ID NO:1) encoding the Ependymin polypeptide having the amino acid sequence in positions -37 to 187 of SEQ ID NO:2.

4.   The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figures 1A, 1B, and 1C (SEQ ID NO:1) encoding the mature Ependymin polypeptide having the amino acid sequence from about 1 to about 187 in SEQ ID NO:2.

5.   An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a)   a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of residues n-187 of SEQ ID NO:2, where n is an integer in the range of -37 to 5;

(b)   a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of residues -37-m of SEQ ID NO:2, where m is an integer in the range of 173 to 187;

(c)   a nucleotide sequence encoding a polypeptide having the amino acid sequence consisting of residues n-m of SEQ ID NO:2, where n and m are integers as defined respectively in (a) and (b) above; and

(d)   a nucleotide sequence encoding a polypeptide consisting of a portion of the complete Ependymin amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209464 wherein said portion excludes from 1 to about 42 amino acids from the amino terminus of said complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209464;

(e) a nucleotide sequence encoding a polypeptide consisting of a portion of the complete Ependymin amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209464 wherein said portion excludes from 1 to about 15 amino acids from the carboxy terminus of said complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209464; and

(f) a nucleotide sequence encoding a polypeptide consisting of a portion of the complete Ependymin amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209464 wherein said portion include a combination of any of the amino terminal and carboxy terminal deletions in (d) and (e), above.

6.   The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in ATCC Deposit No. 209464.

7.   The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the Ependymin polypeptide having the complete amino acid sequence excepting the N-terminal methionine encoded by the cDNA clone contained in ATCC Deposit No. 209464.

8.   The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the mature Ependymin polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209464.

9.   An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e), (f) or (g) of claim 1 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

10.   An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a Ependymin polypeptide having an amino acid sequence in (a), (b), (c), (d), (e) or (f) of claim 1.

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11.   The isolated nucleic acid molecule of claim 10, which encodes an epitope-bearing portion of a Ependymin polypeptide wherein the amino acid sequence of said portion is selected from the group of sequences in SEQ ID NO:2 consisting of: about Ala-1 to about Gln-9; about Pro-8 to about Val-16; about Gln-19 to about Arg-27; about Ile-69 to about Ser-77; about Asp-86 to about Glu-107; about Glu-113 to about Tyr-123; about Thr-131 to about Gln-139; about Leu-159 to about Phe-167; and about Leu-178 to about Ser-186.

12.   A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

13.   A recombinant vector produced by the method of claim 12.

14.   A method of making a recombinant host cell comprising introducing the recombinant vector of claim 13 into a host cell.

15.   A recombinant host cell produced by the method of claim 14.

16.   A recombinant method for producing a Ependymin polypeptide, comprising culturing the recombinant host cell of claim 15 under conditions such that said polypeptide is expressed and recovering said polypeptide.

17.   An isolated Ependymin polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the amino acid sequence of the full-length Ependymin polypeptide having the complete amino acid sequence shown in SEQ ID NO:2 (i.e., positions -37 to 187 of SEQ ID NO:2);

(b) the amino acid sequence of the full-length Ependymin polypeptide having the complete amino acid sequence shown in SEQ ID NO:2 excepting the N-terminal methionine (i.e., positions -36 to 187 of SEQ ID NO:2);

(c) the amino acid sequence of the predicted mature Ependymin polypeptide having the amino acid sequence at positions 1 to 187 in SEQ ID NO:2;

(d) the complete amino acid sequence encoded by the cDNA clone contained in the ATCC Deposit No. 209464;

(e) the complete amino acid sequence excepting the N-terminal methionine encoded by the cDNA clone contained in the ATCC Deposit No. 209464; and

(f) the complete amino acid sequence of the predicted mature Ependymin polypeptide encoded by the cDNA clone contained in the ATCC Deposit No. 209464.

18.   An isolated polypeptide comprising an epitope-bearing portion of the Ependymin protein, wherein said portion is selected from the group consisting of: a polypeptide comprising amino acid residues from about Ala-1 to about Gln-9 in SEQ ID NO:2; a

polypeptide comprising amino acid residues from about Pro-8 to about Val-16 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Gln-19 to about Arg-27 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Ile-69 to about Ser-77 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Asp-86 to about Glu-107 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Glu-113 to about Tyr-123 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Thr-131 to about Gln-139 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about Leu-159 to about Phe-167 in SEQ ID NO:2; and a polypeptide comprising amino acid residues from about Leu-178 to about Ser-186 in SEQ ID NO:2.

19.   An isolated antibody that binds specifically to a Ependymin polypeptide of claim 17.

21.   An isolated polynucleotide encoding a modified Ependymin protein, wherein, except for at least one conservative amino acid substitution, said modified peptide has an amino acid sequence that is identical to a member selected from the group consisting of:

(a) amino acids -37 to 187 of SEQ ID NO:2;

(b) amino acids -36 to 187 of SEQ ID NO:2; and

(c) amino acids 1 to 187 of SEQ ID NO:2.

22.   A modified Ependymin, wherein, except for at least one conservative amino acid substitution, said modified peptide has an amino acid sequence that is identical to a member selected from the group consisting of:

(a) amino acids -37 to 187 of SEQ ID NO:2;

(b) amino acids -36 to 187 of SEQ ID NO:2; and

(c) amino acids 1 to 187 of SEQ ID NO:2.

23.   An isolated nucleic acid molecule comprising a polynucleotide having a sequence at least 95% identical to a sequence selected from the group consisting of:

(a)   the nucleotide sequence of SEQ ID NO:15;

(b)   the nucleotide sequence of SEQ ID NO:16;

(c)   the nucleotide sequence of SEQ ID NO:17;

(d)   the nucleotide sequence of SEQ ID NO:18;

(e)   the nucleotide sequence of SEQ ID NO:19;

(f)   the nucleotide sequence of SEQ ID NO:20;

(g)   the nucleotide sequence of a portion of the sequence shown in Figures 1A, 1B, and 1C (SEQ ID NO:1) wherein said portion comprises at least 50 contiguous nucleotides from nucleotide 1 to nucleotide 2505; and

(h)   a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f) or (g), above.

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24.   A method for the treatment of a patient having need of human ependymin polypeptide comprising:

(a)   isolating the polypeptide of claim 1, and

(b)   administering to the patient a therapeutically effective amount of said polypeptide.

25.   A method for identifying compounds which bind to and inhibit activation of the polypeptide of claim 1 comprising:

(a)   contacting a cell expressing on the surface thereof a receptor for the polypeptide, said receptor being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said receptor, with an analytically detectable human cytokine polypeptide and a compound under conditions to permit binding to the receptor; and

(b)   determining whether the compound binds to and inhibits the receptor by detecting the absence of a signal generated from the interaction of the human ependymin polypeptide with the receptor.

27.   A process for diagnosing a disease or a susceptibility to a disease related to an under-expression of the polypeptide of claim 1 comprising:

(a)   isolating a sample of the nucleic acid sequence encoding said polypeptide, and

(b)   determining a mutation in a nucleic acid sequence encoding said polypeptide.

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Human Ependymin

```
  1.  CCACGCGTCCGGAAAAACCGAAGCGGCAGAAGGCAGTGGCAGCAGGCAGTGGCCCAGGCAG   60

 61   AAATAGCTCCCGCGCGATTCACTGGAGCCTTCCCCGGGCCCTGGTCCCGGCTACCGGGAC   120

121   TCGCGCGTCCGGATCTCAAAAGCGGCAGAGGCCACCGAAGGGACAGGAAGCACTTTGGTC   180

181   CAGACCACACTCCCGGCACAGTGCGGAAAGAGCCGGCGGGAGCCACTCTGATCCCGGACG   240

241   CCTCAGCGCCCCCTTGGGCTTGGGCTTGCCCTCGGGCCGGGGAAGGCTGACCGCGATGCC   300
  1                                                               M  P     2

301   AGGACGCGCTCCCCTCCGCACCGTCCCGGGCGCCCTGGGTGCCTGGCTGCTGGGCGGCCT   360
  3    G  R  A  P  L  R  T  V  P  G  A  L  G  A  W  L  L  G  G  L    22
                                                 CD-I

361   CTGGGCCTGGACCCTGTGCGGCCTGTGCAGCCTGGGGGCGGTGGGAGCCCCGCGCCCGTG   420
 23    W  A  W  T  L  C  G  L  C  S  L  G  A  V  G  A  P  R  P  C    42
            CD-I                                        CD-II

421   CCAGGCGCCGCAGCAGTGGGAGGGGCGCCAGGTTATGTACCAGCAAAGTAGCGGGCGCAA   480
 43    Q  A  P  Q  Q  W  E  G  R  Q  V  M  Y  Q  Q  S  S  G  R  N    62
       CD-II

481   CAGCCGCGCCCTGCTCTCCTACGACGGGCTCAACCAGCGCGTGCGGGTGCTGGACGAGAG   540
 63    S  R  A  L  L  S  Y  D  G  L  N  Q  R  V  R  V  L  D  E  R    82
                      CD-III

541   GAAGGCGCTGATCCCCTGCAAGAGATTATTTGAATATATTTTGCTGTATAAGGATGGAGT   600
 83    K  A  L  I  P  C  K  R  L  F  E  Y  I  L  L  Y  K  D  G  V    102
                                                            CD-IV

601   GATGTTTCAGATTGACCAAGCCACCAAGCAGTGCTCAAAGATGACCCTGACACAGCCCTG   660
103    M  F  Q  I  D  Q  A  T  K  Q  C  S  K  M  T  L  T  Q  P  W    122
       CD-IV                          CD-V

                            #
661   GGATCCTCTTGACATTCCTCAAAACTCCACCTTTGAAGACCAGTACTCCATTGGGGGGCC   720
123    D  P  L  D  I  P  Q  N  S  T  F  E  D  Q  Y  S  I  G  G  P    142
              CD-VI

721   TCAGGAGCAGATCACCGTCCAGGAGTGGTCGGACAGAAAGTCAGCTAGATCCTATGAAAC   780
143    Q  E  Q  I  T  V  Q  E  W  S  D  R  K  S  A  R  S  Y  E  T    162
       CD-VII

                                                          #.
781   CTGGATTGGCATCTATACAGTCAAGGATTGCTATCCTGTCCAGGAAACCTTTACCATAAA   840
163    W  I  G  I  Y  T  V  K  D  C  Y  P  V  Q  E  T  F  T  I  N    182
                CD-VIII
```

FIG. 1A

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Human Ependymin

```
841  CTACAGTGTGATATTGTCTACGCGGTTTTTTGACATCCAGCTGGGTATTAAAGACCCCTC  900
183   Y  S  V  I  L  S  T  R  F  F  D  I  Q  L  G  I  K  D  P  S   202
                  CD-IX                                  CD-X
```

```
901  GGTGTTTACCCCTCCAAGCACGTGCCAGATGGCCCAACTGGAGAAGATGAGCGAAGACTG  960
203   V  F  T  P  P  S  T  C  Q  M  A  Q  L  E  K  M  S  E  D  C  222
                  CD-X
```

```
961  CTCCTGGTGAGCCTGTGCATAGGGAAGCGGCAGCATCGGATGTCAGCCCCCTGCGGCCCC  1020
223   S  W                                                         224
```

```
1021  AGCTGGAGATGGATATGAGACTAGTCAAGATGTGAATGCTAATTGGAGAGAAATATAATT  1080
```

```
1081  TTAGGAAGATGCACATTGATGTGGGGTTTTGATGTGTCTGATTTTGACTACTCAAGCTCT  1140
```

```
1141  GTTTACAGAAGAAAATTGAATGGCGAGGGTGTGGCCATATGAACTGACTAGATGGCTAAT  1200
```

```
1201  ATGGACACTTTGGGTATTTCTAATGCCTGTTCAGGGCTGGTTTTCTGCATGCACGGGTAT  1260
```

```
1261  ACACATAATGCAGTGCCATGCACATAGGGAAGGGTCAGTAAGAGAAGTTTGCCTTGGCAG  1320
```

```
1321  CAAGTATTTATTGTTGACATTATTCAGAATTAGTGATAATAAAAAGCAGAGTGATTTTGG  1380
```

```
1381  TCAATTTTATTATTAATTCTTAAATTCCCTGCAGAGAATGCCCCCTTTATTGCTGCACCA  1440
```

```
1441  GGGTGGGCATTGCTCCCACTGAGCCCTACTCCACCCTGTCCCTGCACTCCCTTGGTTGCC  1500
```

```
1501  AAAAAAATGATAACTTAAATCCCTTCCAGACTTAAGAATTTTATGGCATGGCCCAATTGA  1560
```

```
1561  TATAAACATTTAGAAGGAAATGAAAAGCTAAAATAGGAAGTAATTATTCCTCTAAAGAAA  1620
```

```
1621  CATTTTGAGCAAGGCAGTTTAGAGAATCCTAATGTCTACACTGGCATAGCACGAGCCATG  1680
```

```
1681  TAAGCTTCTTTTTTTTTCTATGCAAGAGTATTGATGTATGTGCTGAATCTTCACAGACTTG  1740
```

```
1741  TCAATACACAGGCAGTATTCTAAAATAGCACTGAACAGGGAGTCAGGAGACTATTGTCTC  1800
```

FIG. 1B

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Human Ependymin

1801 CTAAACCCAGGACTAGAGTTCCCTCGTACTGTCACTCCTTTGGTCATTAAATGCACTGGG 1860

1861 CTTGCCCGCACTTTGGCCTTCCTAGAACGCTGCTTCATAACCTCTCTGTCTGACTTCTGC 1920

1921 ATCTCCTTCCAGGTCAGCTCATTCACAAGAGTTGCTCCCAAGCCTGGATGAGTTGCACCT 1980

1981 TGCATCTTGAGCATGCATTTCTCACAATAATTATTAAGCTGTGTGATAATTTCTGCTTTC 2040

2041 AGGACACTCATCCATTATCTTGGCTGTGAGCTCCTTGGGTACGGGTACCTTGTATGTTTA 2100

2101 ATTTTATATCCCTAGCACAAAGCAAGTGCCTGGCACATAGTCAGTGCCCTAAGTATTTGT 2160

2161 AGAGTGAAGAATGCCAGCCTCTCTTGTCCCTGGTTTCCTTATGTGTTGAATGTGGTTGAG 2220

2221 TTTGTCCATTGCTAGGGAGAGACTTCCAGTAATAAAATTTACTATTCTAGATGCTTCTAC 2280

2281 TGTTATGTTTTATCTGCCCATTTATCTTTCTTAGTTACCAGGAGAAATGTGTGACACCTA 2340

2341 TATTATAATGAAAACAATCTTATTACTTATAGTTTATCTATATTAAACAAATTTAATTGC 2400

2401 ATTTAAAGCATTCTTTGATATTGTTGCTTTTGCAATAAATATGGATAATCTTGGTTATAA 2460

2461 GGGAGTTAAAACAATGCTGTAATA<u>AATAAA</u>GTGTTTCATGTGATCAAAAAAAAAAAAAAAA 2520

2521 AAAAA 2525

FIG. 1C

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Human Ependymin vs. Zebrafish Ependymin

Percent Similarity: 36.813   Percent Identity: 22.527

```
            .         .         .         .         .
 40 RPCQAPQQWEGRQVMYQQSSGRNSRALLSYDGLNQRVRVLDERKALIPCK 89
    .||.||    |    .           |||      : |  .::
 27 QPCHSPQLTSGTMKVVSTGGHDLASGEFSYDSKTNKFRFVEDTTHANKTS 76

            .         .         .         .         .
 90 RLFEYILLYKDGVMFQIDQATKQCSKMTLTQPWDPLDIPQNSTFEDQYSI 139
    : : :: :.:||:::||   . | | ||      ::|| ..| | :  .
 77 YI.DVLIHFEEGVLYEIDSKNESCKKETLQFRKHLMEIPVDATHESESYM 125

            .         .         .         .         .
140 GGP...QEQITVQEWSDRKSARSYETWIGIYTVKDCYPVQETFTINYSVI 186
    | |   :: : |. |. |    :  :    | | |   .:
126 GSPSLTEQGLRVRVWNG.KFPELHAHYSLSTTSCGCLTVSGSY.YGEKKD 173

            .         .         .
187 LSTRFFDIQLGIKDPSVFTPPSTCQMAQLEKMSEDCSW 224
    |   || ::   : |  || ||. |:    |. :| |.
174 LFFSFFGVETEVDDLQVFAPPAYCEGVSFEEAPDDHSF 211
```

FIG. 2

FIG.3A

FIG.3B

```
                110        120        130        140
121  PWCPLDIPQNSTFFEDQYSIGGP---QEQITVQEWSDRKSA   huEpendymin.aa
108  HAHALELPAGAAHQVELFLGSDTVQEDNIKVNIWMGSVAE   rainb.troutEPD-II.aa
107  RKHLMEIPVDATHESESYMGSPSLTEQQGLRWVNGKFPE   zebrafishEPN.aa
103  TYHCLEVPPNATHVTEGYLGSEFIGDQGVRMRKRVPE     atl.herringEPN.aa
105  RKHLMEIPPDATHESEIYMGSFSITEQGLRVRVWNGKLPE  carpEPN.aa
106  RKHLMEIPPDATHESEIYMGSFSITEQGLRVRVWSGKLPE  goldfishEPN-IIprec.aa

                150        160        170        180
158  RSYETWIGIVTVKDCYPVQETFTINYSVILSTRFFDIQLG   huEpendymin.aa
148  TKGQYSALT-TVGECLPLS-TFYSTDSITLLFSNSEVVTE   rainb.troutEPD-II.aa
147  LHAHYSLST-TSCGCLTVSGSYYGEKKDLF-FSFFGVETE  zebrafishEPN.aa
143  LDGVVTVAT-TSCGCVTLFATLFTDSNDVLVFNFLDVEMK  atl.herringEPN.aa
145  LHAHYSLST-TSCGCLPVSGSYYGDKKDLL-FSFFGVETE  carpEPN.aa
146  LHAHYSLST-TSCGCLPVSGSYYGDKKDLL-FSFFGVETE  goldfishEPN-IIprec.aa

                190        200        210
198  LKDP-SVFTPPSTCQMAQLEKMSEDCSW.            huEpendymin.aa
186  VKAP-EMFTLPSFCEAVELEETPKGQKNDFFNIFNTV    rainb.troutEPD-II.aa
185  VDD-LQVFAPPAYCEGVSFEEAPDDH--SFFDLFHD     zebrafishEPN.aa
182  VKNFLEVFVPPSYCDGVALE--EGD--TFFGLFH       atl.herringEPN.aa
183  VDD-PQVFVPPAYCEAVAFEEAPDDH--SFFDLFHD     carpEPN.aa
184  VDD-LQVFVPPAYCEGVAFEEAPDDH--SFFDLFHD     goldfishEPN-IIprec.aa
```

Protein Analysis of Human Ependymin

FIG. 4

1
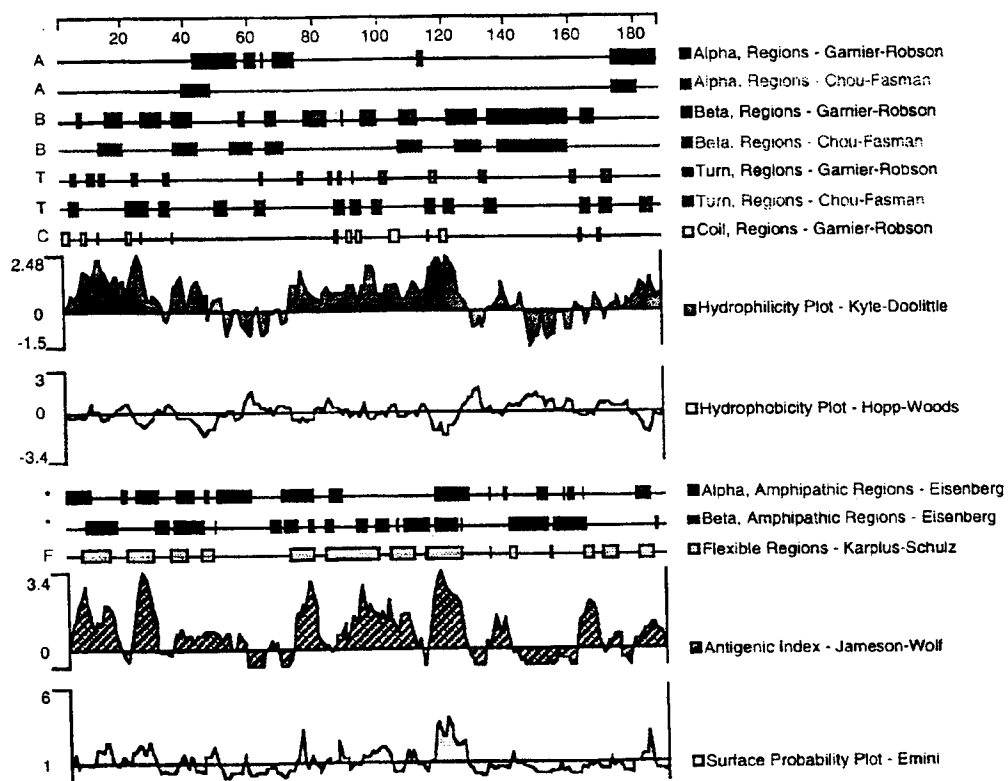
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      Ruben M., Steven

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tcgcgcgtcc ggatctcaaa agcggcagag gccaccgaag ggacaggaag cactttggtc 180

cagaccacac tcccggcaca gtgcggaaag agccggcggg agccactctg atcccggacg 240

cctcagcgcc cccttgggct tgggcttgcc ctcgggccgg ggaaggctga ccgcg atg   298
                                                                 Met

cca gga cgc gct ccc ctc cgc acc gtc ccg ggc gcc ctg ggt gcc tgg   346
Pro Gly Arg Ala Pro Leu Arg Thr Val Pro Gly Ala Leu Gly Ala Trp
    -35   -30   -25

ctg ctg ggc ggc ctc tgg gcc tgg acc ctg tgc ggc ctg tgc agc ctg   394
Leu Leu Gly Gly Leu Trp Ala Trp Thr Leu Cys Gly Leu Cys Ser Leu
-20   -15   -10   -5

ggg gcg gtg gga gcc ccg cgc ccg tgc cag gcg ccg cag cag tgg gag   442
Gly Ala Val Gly Ala Pro Arg Pro Cys Gln Ala Pro Gln Gln Trp Glu
              -1   1   5   10

```
ggg cgc cag gtt atg tac cag caa agt agc ggg cgc aac agc cgc gcc    490
Gly Arg Gln Val Met Tyr Gln Gln Ser Ser Gly Arg Asn Ser Arg Ala
        15                  20                  25

ctg ctc tcc tac gac ggg ctc aac cag cgc gtg cgg gtg ctg gac gag    538
Leu Leu Ser Tyr Asp Gly Leu Asn Gln Arg Val Arg Val Leu Asp Glu
    30                  35                  40

agg aag gcg ctg atc ccc tgc aag aga tta ttt gaa tat att ttg ctg    586
Arg Lys Ala Leu Ile Pro Cys Lys Arg Leu Phe Glu Tyr Ile Leu Leu
45                  50                  55                  60

tat aag gat gga gtg atg ttt cag att gac caa gcc acc aag cag tgc    634
Tyr Lys Asp Gly Val Met Phe Gln Ile Asp Gln Ala Thr Lys Gln Cys
                65                  70                  75

tca aag atg acc ctg aca cag ccc tgg gat cct ctt gac att cct caa    682
Ser Lys Met Thr Leu Thr Gln Pro Trp Asp Pro Leu Asp Ile Pro Gln
            80                  85                  90

aac tcc acc ttt gaa gac cag tac tcc att ggg ggg cct cag gag cag    730
Asn Ser Thr Phe Glu Asp Gln Tyr Ser Ile Gly Gly Pro Gln Glu Gln
            95                  100                 105

atc acc gtc cag gag tgg tcg gac aga aag tca gct aga tcc tat gaa    778
Ile Thr Val Gln Glu Trp Ser Asp Arg Lys Ser Ala Arg Ser Tyr Glu
    110                 115                 120

acc tgg att ggc atc tat aca gtc aag gat tgc tat cct gtc cag gaa    826
Thr Trp Ile Gly Ile Tyr Thr Val Lys Asp Cys Tyr Pro Val Gln Glu
125                 130                 135                 140

acc ttt acc ata aac tac agt gtg ata ttg tct acg cgg ttt ttt gac    874
Thr Phe Thr Ile Asn Tyr Ser Val Ile Leu Ser Thr Arg Phe Phe Asp
                145                 150                 155

atc cag ctg ggt att aaa gac ccc tcg gtg ttt acc cct cca agc acg    922
Ile Gln Leu Gly Ile Lys Asp Pro Ser Val Phe Thr Pro Pro Ser Thr
            160                 165                 170

tgc cag atg gcc caa ctg gag aag atg agc gaa gac tgc tcc tgg        967
Cys Gln Met Ala Gln Leu Glu Lys Met Ser Glu Asp Cys Ser Trp
        175                 180                 185
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ctttttttc tatgcaagag tattgatgta tgtgctgaat cttcacagac ttgtcaatac 1747

acaggcagta ttctaaaata gcactgaaca gggagtcagg agactattgt ctcctaaacc 1807

caggactaga gttccctcgt actgtcactc ctttggtcat taaatgcact gggcttgccc 1867

gcactttggc cttcctagaa cgctgcttca taacctctct gtctgacttc tgcatctcct 1927

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agaatgccag cctctcttgt ccctggtttc cttatgtgtt gaatgtggtt gagtttgtcc 2227

attgctaggg agagacttcc agtaataaaa tttactattc tagatgcttc tactgttatg 2287

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Trp Leu Leu Gly Gly Leu Trp Ala Trp Thr Leu Cys Gly Leu Cys Ser
      -20               -15               -10

Leu Gly Ala Val Gly Ala Pro Arg Pro Cys Gln Ala Pro Gln Gln Trp
  -5                -1   1                 5                  10

Glu Gly Arg Gln Val Met Tyr Gln Gln Ser Ser Gly Arg Asn Ser Arg
              15                20                25

Ala Leu Leu Ser Tyr Asp Gly Leu Asn Gln Arg Val Arg Val Leu Asp
        30                35                40

Glu Arg Lys Ala Leu Ile Pro Cys Lys Arg Leu Phe Glu Tyr Ile Leu
        45                50                55

Leu Tyr Lys Asp Gly Val Met Phe Gln Ile Asp Gln Ala Thr Lys Gln
  60                65                70                75

Cys Ser Lys Met Thr Leu Thr Gln Pro Trp Asp Pro Leu Asp Ile Pro
                80                85                90

Gln Asn Ser Thr Phe Glu Asp Gln Tyr Ser Ile Gly Gly Pro Gln Glu
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4

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                95                    100                   105
Gln Ile Thr Val Gln Glu Trp Ser Asp Arg Lys Ser Ala Arg Ser Tyr
        110                   115                   120

Glu Thr Trp Ile Gly Ile Tyr Thr Val Lys Asp Cys Tyr Pro Val Gln
    125                   130                   135

Glu Thr Phe Thr Ile Asn Tyr Ser Val Ile Leu Ser Thr Arg Phe Phe
140                   145                   150                   155

Asp Ile Gln Leu Gly Ile Lys Asp Pro Ser Val Phe Thr Pro Pro Ser
                160                   165                   170

Thr Cys Gln Met Ala Gln Leu Glu Lys Met Ser Glu Asp Cys Ser Trp
            175                   180                   185


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<212> PRT
<213> Goldfish

<400> 3
Met Met His Thr Val Lys Leu Leu Cys Val Val Phe Ser Cys Leu Cys
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Ala Val Ala Trp Ala Ser Ser Asp Arg Gln Pro Cys His Ser Pro Pro
            20                    25                    30

Leu Ile Ser Gly Thr Met Lys Val Val Ser Thr Gly Gly His Asp Leu
         35                    40                    45

Ala Ser Gly Glu Phe Ser Tyr Asp Ser Lys Ala Asn Lys Phe Arg Phe
     50                    55                    60

Val Glu Asp Ala Ala His Ala Asn Lys Thr Ser His Thr Asp Val Leu
65                    70                    75                    80

Val His Phe Glu Glu Gly Thr Leu Tyr Glu Ile Asp Ser Lys Asn Glu
                85                    90                    95

Ser Cys Lys Lys Glu Thr Leu Gln Phe Arg Lys His Leu Met Glu Ile
            100                   105                   110

Pro Pro Asp Ala Thr His Glu Ser Glu Ile Tyr Met Gly Ser Pro Ser
         115                   120                   125

Ile Thr Glu Gln Gly Leu Arg Val Arg Val Trp Ser Gly Lys Leu Pro
     130                   135                   140

Glu Leu His Ala His Tyr Ser Leu Ser Ile Thr Ser Cys Gly Cys Leu
145                   150                   155                   160

Pro Val Ser Gly Ser Tyr Tyr Gly Asp Lys Lys Asp Leu Leu Phe Ser
            165                   170                   175

Phe Phe Gly Val Glu Thr Glu Val Asp Asp Leu Gln Val Phe Val Pro
         180                   185                   190

Pro Ala Tyr Cys Glu Gly Val Ala Phe Glu Glu Ala Pro Asp Asp His
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Ser Phe Phe Asp Leu Phe His Asp
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<212> PRT
<213> Rainbow Trout

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Met Gln Asp Phe Ala Phe Ala Ala Leu Ser Ile Trp Leu Cys Leu Gly
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Asn Met Thr Gly Val Leu Thr Val Met Ala Leu Thr Gly Gly Glu Ile
          35   40   45

Lys Ala Thr Gly His Tyr Ser Tyr Asp Ser Thr Asn Lys Lys Leu Arg
    50   55   60

Phe Thr Glu Ser Glu Met His Leu Asn Lys Thr Glu His Leu Glu Asp
65   70   75   80

Tyr Leu Met Leu Phe Glu Glu Gly Val Phe Tyr Asp Ile Asp Met Lys
                85   90   95

Asn Gln Ser Cys Lys Lys Met Ser Leu His Ser His Ala His Ala Leu
            100   105   110

Glu Leu Pro Ala Gly Ala Ala His Gln Val Glu Leu Phe Leu Gly Ser
          115   120   125

Asp Thr Val Gln Glu Asp Asn Ile Lys Val Asn Ile Trp Met Gly Ser
    130   135   140

Val Ala Glu Thr Lys Gly Gln Tyr Ser Ala Leu Thr Thr Val Gly Glu
145   150   155   160

Cys Leu Pro Leu Ser Thr Phe Tyr Ser Thr Asp Ser Ile Thr Leu Leu
                165   170   175

Phe Ser Asn Ser Glu Val Val Thr Glu Val Lys Ala Pro Glu Met Phe
            180   185   190

Thr Leu Pro Ser Phe Cys Glu Ala Val Glu Leu Glu Glu Thr Pro Lys
          195   200   205

Gly Gln Lys Asn Asp Phe Phe Asn Ile Phe Asn Thr Val
    210   215   220

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<211> 215
<212> PRT
<213> Common Carp

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Val Ala Trp Ala Ser Ser Asn Arg Gln Pro Cys His Ser Pro Pro Leu

6

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                20                  25                  30
Thr Ser Gly Thr Met Lys Val Val Ser Thr Gly Gly His Asp Leu Ala
        35                  40                  45

Ser Gly Glu Phe Ser Tyr Asp Ser Lys Ala Asn Lys Phe Arg Phe Val
    50                  55                  60

Glu Asp Thr Ala His Ala Asn Lys Thr Ser His Met Asp Val Leu Val
65                  70                  75                  80

His Phe Glu Glu Gly Val Leu Tyr Glu Ile Asp Ser Lys Asn Glu Ser
                85                  90                  95

Cys Lys Lys Glu Thr Leu Gln Phe Arg Lys His Leu Met Glu Ile Pro
            100                 105                 110

Pro Asp Ala Thr His Glu Ser Glu Ile Tyr Met Gly Ser Pro Ser Ile
        115                 120                 125

Thr Glu Gln Gly Leu Arg Val Arg Val Trp Asn Gly Lys Leu Pro Glu
    130                 135                 140

Leu His Ala His Tyr Ser Leu Ser Thr Thr Ser Cys Gly Cys Leu Pro
145                 150                 155                 160

Val Ser Gly Ser Tyr Tyr Gly Asp Lys Lys Asp Leu Leu Phe Ser Phe
                165                 170                 175

Phe Gly Val Glu Thr Glu Val Asp Asp Pro Gln Val Phe Val Pro Pro
            180                 185                 190

Ala Tyr Cys Glu Ala Val Ala Phe Glu Glu Ala Pro Asp Asp His Ser
        195                 200                 205

Phe Phe Asp Leu Phe His Asp
    210                 215


<210> 6
<211> 217
<212> PRT
<213> Zebrafish

<400> 6
Met His Thr Val Lys Leu Leu Cys Val Val Phe Ser Cys Leu Cys Ala
  1               5                  10                  15

Ile Gly Trp Ala Ser His His Ser His Arg Gln Pro Cys His Ser Pro
            20                  25                  30

Gln Leu Thr Ser Gly Thr Met Lys Val Val Ser Thr Gly Gly His Asp
        35                  40                  45

Leu Ala Ser Gly Glu Phe Ser Tyr Asp Ser Lys Thr Asn Lys Phe Arg
    50                  55                  60

Phe Val Glu Asp Thr Thr His Ala Asn Lys Thr Ser Tyr Ile Asp Val
65                  70                  75                  80

Leu Ile His Phe Glu Glu Gly Val Leu Tyr Glu Ile Asp Ser Lys Asn
                85                  90                  95
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Glu Ser Cys Lys Lys Glu Thr Leu Gln Phe Arg Lys His Leu Met Glu
            100                 105                 110

Ile Pro Val Asp Ala Thr His Glu Ser Glu Ser Tyr Met Gly Ser Pro
            115                 120                 125

Ser Leu Thr Glu Gln Gly Leu Arg Val Arg Val Trp Asn Gly Lys Phe
        130                 135                 140

Pro Glu Leu His Ala His Tyr Ser Leu Ser Thr Thr Ser Cys Gly Cys
145                 150                 155                 160

Leu Thr Val Ser Gly Ser Tyr Tyr Gly Glu Lys Lys Asp Leu Phe Phe
                165                 170                 175

Ser Phe Phe Gly Val Glu Thr Glu Val Asp Asp Leu Gln Val Phe Ala
            180                 185                 190

Pro Pro Ala Tyr Cys Glu Gly Val Ser Phe Glu Glu Ala Pro Asp Asp
            195                 200                 205

His Ser Phe Phe Asp Leu Phe His Asp
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<213> Atlantic Herring

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Met Arg Leu Thr Gly Leu Leu Cys Val Ala Leu Trp Ser Ala Ser Ala
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Val Val Leu Ala Glu His Gln Pro Cys Arg Pro Pro Pro Gln Thr His
            20                  25                  30

Gly Asn Leu Trp Val Thr Ala Ala Lys Gly Ala Pro Ala Ser Val Gly
        35                  40                  45

Glu Phe Asn Tyr Asp Ser Gln Ala Arg Lys Leu His Phe Lys Asp Asp
    50                  55                  60

Ala Leu His Val Asn Lys Thr Asp His Leu Glu Met Leu Ile Phe Phe
65                  70                  75                  80

Glu Glu Gly Ile Phe Tyr Asp Ile Asp Ser His Asn Gln Ser Cys His
                85                  90                  95

Lys Lys Thr Leu Gln Ser Thr Tyr His Cys Leu Glu Val Pro Pro Asn
            100                 105                 110

Ala Thr His Val Thr Glu Gly Tyr Leu Gly Ser Glu Phe Ile Gly Asp
            115                 120                 125

Gln Gly Val Arg Met Arg Lys Trp Arg Lys Arg Val Pro Glu Leu Asp
        130                 135                 140

Gly Val Val Thr Val Ala Thr Thr Ser Cys Gly Cys Val Thr Leu Phe
145                 150                 155                 160

Ala Thr Leu Phe Thr Asp Ser Asn Asp Val Leu Val Phe Asn Phe Leu
                165                 170                 175
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Asp Val Glu Met Lys Val Lys Asn Pro Leu Glu Val Phe Val Pro Pro
            180   185   190

Ser Tyr Cys Asp Gly Val Ala Leu Glu Glu Glu Gly Asp Thr Phe Phe
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Gly Leu Phe His
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<213> Homo sapiens

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<211> 29
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<400> 9
gcgggtacct caccaggagc agtcttcgc   29

<210> 10
<211> 51
<212> DNA
<213> Homo sapiens

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<212> DNA
<213> Homo sapiens

<400> 11
gatcgcggta ccttatcacc aggagcagtc ttcgctcatc ttctccag   48

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<211> 42
<212> DNA
<213> Homo sapiens

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gatcgcggta ccgccatcat gccaggacgc gctcccctcc gc   42

<210> 13
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<212> DNA
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<400> 13
gatcgcggat cctcaccagg agcagtcttc gc   32

9

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<212> DNA
<213> Homo sapiens

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gcatgcattt ctcacaataa ttattaagct gtgtgataat ttctgctttc aggacactca   120

tccattatct tggctgtgag ctccttgggt acgggtacct tgtatgttta attttatatc   180
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cctagcacaa agcaagtgcc tggcacatag tcagtgccct aagtatttgt agagtgaaga 240

atgccagcct ctcttgtccc tggtttcctt atgtgttgaa tgtggttgag tttgtccatt 300

gctagggaga gacttccagt aataaaattt actattctag atgntctact gtantgttta 360

tctgcccatt tatcttctta gtaccagggg aaagtgtgnc accctttttt aatggnaaca 420

acttnttacc taaggttacc cttttaacaa attaatgcat taagnatctt ggaatgtggc 480

ttgaaaaaan gggaactggt nnaggg                                      506
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taagcttctt tttttctat gcaagagtat tgatgtatgt nctgaatctt cacagacttg 120

tcaatacaca ggcagtattc taaaatagca ctgaacaggg agtcaggaga ctattgtctc 180

ctaaacccag gactagagtt ccctcgtact gtcactcctt tggtcattaa atgcactggg 240

cttgcccgca ctttggcctt cctagaacgt ngnttcaaaa cctntttggt ctgacttttg 300

naattccctt ccagggnang tcattcanaa ggggtttttc caagcctngg tgggttnaac 360

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12

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aaaagcagag tgattttggt caattttatt attaattctt aaattccctg cagagaatgc 120

cccctttatt gctgcaccag ggtgggcatt gctcccactg agccctactc caccctgtcc 180

ctgcactccc ttggttgcca aaaaaatgat aacttaaatc ccttccagac ttaagaattt 240

tatgggcatg gnccaattga tattaaacat ttagaaggga atgaaagctt aaatagggag 300

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cccttataac caagattatc catatttatt gcaaaagcaa caatatcaaa gaatgcttta 120

aaatgcaatt gaaatttgtt taatatagat aaactataag taataagatt gttttcatta 180

taatataggt gtcacacatt tctcctggta actaaggaaa gataaatggg cagataaaac 240

ataacagtag aagcatctag gattngttaa tttttnttac tgggagngct cttccctagg 300

cattttacca acttcaacca ctttcaacac ataggggaac ccagggccca ggggggggctg 360

ggcatttttc antttggggg ttnnttgggg cccggcnttt ntgcccgggn ttgnttntgg 420

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nnnccaggct ggacgagagg aaggcgctga tcccctgcaa gagaattatt tggatatatt 120

ttggtgtata aggatggagt gatgtttcaa attgaccaag ccaccaagca gtgctcaaag 180

ccctgacaca gccctgggat cctcttgaca ttcctcaaaa ctccaccttt gaagaccagt 240

actccattgg ggggcctcag gagccagntc accgtccagn agtggtcgga cagaaagtca 300

gcttaggtcc tatgaaacct gggttgggca tttatnacag ttcaaggntt ggttnttctg 360

ttccagnaaa ccttttacct nt                                           382


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15

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tctttgatat tgttgctttt gcaatgaaat atggataatc ttggttataa gggagttaaa 120

acaatgctgt aataaataaa gtgtttcatg tgatcaaaat cannttttgtt atcctctctc 180

aaaagcctgc tctccctctt accttcccta aactccagtg tccatttgcc aaggccagaa 240

gcttccaagt cattcttgga tccatttgtc tccctcagtc ctcactgggc agtncaatat 300

cccgagtcct attcaaaata gcttctttaa aactttntgc ctgtttcttc cactnccttg 360

nccctcaggc cagggggccct gctgggaagg gggctggcat ntttcctgtt cagncctggg 420

gtttnccatg ggtggactgg gngtgacgga ggccacctgg nggtt           465
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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6)   :C12P 21/00; C12N 15/12; C07K 14/47
US CL   :435/69.1; 536/23.5; 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   435/69.1, 71.1, 471, 325, 252.3; 536/23.5, 24.31; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ADAMS, et al. Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence. Nature. 28 September 1995, Vol 377 (6547 Supp), pages 3-17, see entire document.	9, 23
A	HOFFMANN, W. Ependymins and their potential role in neuroplascity and regeneration: Calcium-Binding Meningeal Glycoproteins of the CereBrospinal Fluid and Extracellular Matrix. International Journal of Biochemistry. 1994, Vol. 26, No. 5, pages 607-619, see entire document.	1-18, 21-23
A	US 5,545,719 A (SHASHOUA) 13 August 1996), see entire document.	1-18, 21-23

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 MARCH 1999	28 APR 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer FOZIA HAMUD
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

Sequence search: protein data bases and DNA data bases.
APS, MEDLINE, CAPLUS
search terms: Ependymin, human, nucleic acid, polypeptide, recombinant, production.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I, claims 1-18,21-23, drawn to an isolated nucleic acid encoding an ependymin polypeptide, a vector, a host cell, a method for producing ependymin and ependymin polypeptide.
Group II, claim 19, drawn to an isolated antibody that binds specifically to an ependymin polypeptide.
Group III, claim 24, drawn to a method of treatment by administering a therapeutically effective amount of ependymin polypeptide.
Group IV, claim 25, drawn to a method for identifying compounds which bind to and inhibit activation of ependymin polypeptide.
Group V, claim 27, drawn to a process for diagnosing a disease by isolating a nucleic acid encoding ependymin and determining a mutation in it.

(NOTE: Claim numbers 20 and 26 are missing).

The inventions listed as Groups I-VI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
pursuant to 37 C.F.R. § 1.475 (d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first-recited product, an isolated nucleic acid encoding an Ependymin polypeptide, a vector, a host cell, a method for producing ependymin and ependymin polypeptide. Further pursuant to 37 C.F.R. § 1.475 (d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.